From monoclonal gammopathy of undetermined significance to symptomatic multiple myeloma: genetic heterogeneity on all levels

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Abstract

Multiple myeloma (MM) is preceded by pre-malignant disease phases of monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma (SMM). The genetic abnormalities found in MM comprise of intrachromosomal translocations, largely involving the IGH locus, copy number abnormalities, somatic mutations and changes in DNA and histone methylation. Many of these genetic lesions are also present in MGUS and SMM but do not result in the clinical symptoms associated with MM. Here we discuss the common abnormalities in these disease phases along with the impact of intraclonal heterogeneity on the future of myeloma biology and treatment.

Learning goals

At the conclusion of this activity, participants should be able to:

- describe the common genetic abnormalities in multiple myeloma;
- know the common somatic mutations in myeloma and targeted therapy options;
- understand the complex subclonal genetic architecture of myeloma.

Introduction

Multiple myeloma is a genetically complex isease that is becoming more common in today's aging population. Myeloma belongs to a group of related paraproteinemias that are characterized by an abnormal clonal plasma cell infiltration in the bone marrow.[1,2] A number of distinct clinical phases of myeloma can be recognized, including monoclonal gammopathy of undetermined significance (MGUS) and asymptomatic or smoldering multiple myeloma (SMM). Both these phases lack the clinical features of myeloma but share some of the genetic features of a myeloma clone.[3] By contrast, symptomatic multiple myeloma (MM) is defined by clinical symptoms

and evidence of organ damage.

A characteristic feature of myeloma cells is the requirement for an intimate relationship with the bone marrow microenvironment, where plasma cells are nurtured in specialized niches that maintain their survival long term.[4-8] However, during the progression of the disease, clonal cells develop the ability to proliferate at sites outside of the bone marrow, manifesting as extra-medullary myeloma (EMM) and plasma cell leukemia (PCL).[9] These cells constitute the end stages in the multistep transformation process from normal to malignant plasma cells. Here we will review the genetics and techniques used to study the events in the process of transformation from MGUS through SMM, MM and finally to PCL. These include the classically studied translocations and hyperdiploidy, copy number abnormalities and, finally, how genome sequencing strategies are identifying new potential targets in somatic mutations and how these can be used to determine the evolutionary course of disease progression.

Translocations

Chromosomal translocations arise when DNA double strand breaks at different sites in the genome are brought together and aberrantly rejoined.[10]

They are common in tumors of the lymphoid lineage because of the 'off target'effects of the normal physiological mechanismsmediating DNA rearrangement at theimmunoglobulin (IGH) locus. Translocationsinto the IGH locus predominantly occur eitherduring recombination activation gene (RAG) complexmediated V(D)J rearrangement, such as in mantle cell lymphoma (t(11;14)),[11] or during class switch recombination (CSR). Inmyeloma, the primary translocations are thought to be generated via abnormal CSR events mediated by activation-induced cytidine deaminase (AID).[12] This concept has been developed and is based on the location of the translocation breakpoints determined in myeloma cell lines and a few primary samples.

Added to this, the myeloma clone is derived from a mature plasma cell that has undergone somatic hypermutation in the germinal center[13] and does not express the RAG complex.

In myeloma, primary aberrant rearrangementsinto the IGH locus are present in up to 40% of cases.[14,15] There are five main translocation partner chromosomes including the t(4;14) (11%), t(6;14) (2%), t(11;14) (15%), t(14;16) (3%) and t(14;20) (1.5%) which result in the overexpression of MMSET and FGFR3, CCND3, CCND1, MAF and MAFB, respectively, and are thought to confer a selective advantage to the clone (**Figure 1**).[16] Although the translocations over-express very different genes, they have in common downstream deregulation of cyclin D genes, which have been grouped together under the Translocation/Cyclin D (TC) classification.[17] In its simplest form, this classification defines groups of myeloma samples based on their expression of CCND1 (t(11;14)), CCND2 (t(4;14),t(14;16) and t(14;20)), and CCND3 (t(6;14)). However, the translocations themselves are not sufficient to cause progression to myeloma. Evidence for this comes from analysis of MGUS, SMM and MM samples in which translocations are detected, but not at the same frequency.[18] For example, the t(14;20) is present in 5% of MGUS samples but only 1.5% of MM samples, and conversely, the t(4;14) is present in 3% of MGUS but rises to 11% in MM samples.

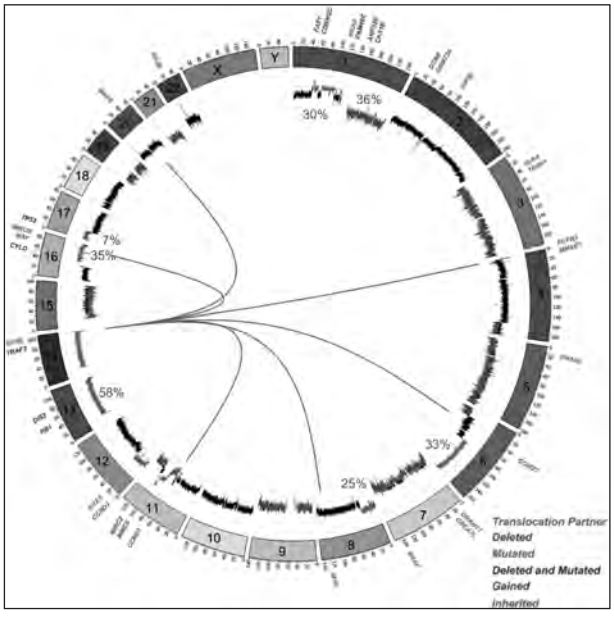

***Figure 1**: The common genetic abnormalities in myeloma. The circos plot shows chromosomes arranged around the outside in a clockwise direction. The internal track shows the common copy number changes with deletions (red) and gains (blue) shown with their frequencies in myeloma. Translocations are indicated by lines across the center between loci. The genes of interest are shown around the outside of the circle and are color-coded according to the legend.*

The conclusions drawn from these data are that some translocations, such as the t(14;20), can be stable in MGUS patients for long periods of time resulting in higher frequencies present in MGUS, whereas the t(4;14) progresses to MM faster, resulting in a lower frequency in MGUS patients.

Copy number changes

In addition to translocations, copy number abnormalities are common in myeloma (**Figure 1**). These abnormalities have been studied by many techniques from karyotyping and fluorescence in situ hybridization (FISH) through to SNP-based mapping arrays, and more recently, exome sequencing. The most prevalent copy number abnormality is the presence of hyperdiploidy, through trisomy of chromosomes 3, 5, 7, 9, 11, 15, 19 and 21, and like the translocations is considered a primary event.

Hyperdiploidy is present in approximately 50% of myeloma samples and is almost mutually exclusive with IGH translocations, where both translocations and hyperdiploidy occur in only 9% of samples. The most commonly gained chromosomes are 9, 15 and 19 but the genetic mechanism of gain and pathogenic advantage still remain elusive. Hyperdiploid patients tend to have a better prognostic outcome than those with IGH translocations. The myeloma genome is rife with additional copy number abnormalities, with almost all chromosomes being affected across samples, indicating genomic instability in myeloma. Aside from the trisomies related to hyperdiploidy, the most common chromosomal abnormalities are del(1p) (30%), 1q+ (36%), del(6q) (33%), del(8p) (25%), 11q+ (24%), del(13q) (58%), del(16q) (35%) and del(17p) (7%).[19] In some of these chromosomes, the genes of interest have been identified but in others they remain elusive. For example, on 1p FAM46C, CDKN2C and FAF1 have been identified as potential targets,[19-21] on 16q CYLD and WWOX are targets of interstitial deletions,[22,23] on 1q CKS1B, ANP32E, BCL9 and PDZK1 have all attracted interest,[19,24,25] and on 17p TP53 is the clear gene of interest.[19,26,27] However, for many of the chromosomal abnormalities (6q, 8p) there is no clear target gene. These last two regions have not been so well studied, in part because they currently have no prognostic value.

Cytogenetic risk stratification

Cytogenetics has been used to determine which genetic lesions have an impact on overall and progression-free survival. Concerning the translocation groups, t(4;14), t(14;16) and t(14;20) are considered to be high risk genetic events resulting in a decreased overall survival.[28]

However, much of the high risk nature of the t(4;14) can be overcome by treatment with bortezomib.[29] t(11;14) and (6;14) are considered standard risk groups, as is hyperdiploidy.

Many of the copy number abnormalities do have a prognostic value in several datasets. In the UK MRC Myeloma IX trial, we have shown del(1p), 1q+ and del(17p) all have an independent statistically significant impact in overall survival.[19,26] This has been confirmed in other datasets with several different treatment contexts.[30-33] Together with t(4;14), these cytogenetic markers have been used to identify patients with high-risk myeloma, which could be managed differently to standard risk patients. One analysis has also determined that the poor prognostic effect of high-risk genetics (t(4;14), t(14;16), t(14;20) or del(17p)) can be ameliorated by the presence of trisomies.[34] Bortezomib administration can also improve outcome in patients with del(17p) when administered before and after autologous stem cell transplantation.[35]

The accumulation of adverse markers has a profound effect on the overall survival of a patient. Many of the adverse lesions co-segregate, so the chance of a patient having more than one abnormality is increased, for example 72% of patients with an IGH translocation also have 1q+. By integrating these known adverse lesions it is possible to more accurately estimate the overall survival of a patient where those without any adverse markers (OS=60.6 months) do better than those with one (OS=41.9 months), two (OS=23.4 months) or three (OS=9.1 months) adverse markers.[36]

Somatic mutations

The most recent developments in myeloma genetics revolve around genome and exome sequencing of samples, allowing the identification of somatic mutations and structural variations. This has been exemplified by the initial publication of the landscape of mutations in myeloma through sequencing

of 38 myeloma samples.[37] The number of non-synonymous (NS) somatic mutations found in myeloma is around 30-35.[37,38] This number is higher than some other hematologic malignancies such as hairy cell leukemia (NS-mutations = 5),[39] acute myeloid leukemia (NS-mutations = 8)[40] but much lower than solid tumors such as lung cancer (NS-mutations = 540).[41] This level of mutation indicates that myeloma is more complex than most hematologic malignancies. The main finding of this initial screen is that there is no unifying mutation in myeloma. In some other hematologic malignancies, a common mutation in most or all samples has been discovered and is thought to be the primary driver mutation. For example, in hairy cell leukemia, the BRAF V600E mutation is found in all samples,[39] and in Waldenströms macroglobulinemia, the MYD88 L265P mutation is found in 91% of samples.[42] In myeloma, the most frequent mutations were found in NRAS (23%) and KRAS (26%), followed by FAM46C (13%, previously identified as deleted and mutated)[19,20] and TP53 (8%). The NRAS and KRAS mutations, with the addition of BRAF mutations (4%), indicates the ERK pathway is critical in at least 53% of myeloma patients and points to a treatment strategy that has so far not been harnessed. ERK pathway mutations are not new to myeloma, but the whole genome strategies have identified some novel mutations not previously identified by other means. These include DIS3 (mutated in 10%) on chromosome 13, a highly conserved RNA nuclease, which is also deleted in 58% of samples. The function of this mutation is not understood, but may be involved in regulation of the available pool of mRNAs available for translation.[43] However, the number of myeloma samples sequenced to date is small and the true landscape of somatic mutations is yet to be realized. As the number of samples sequenced increases, it will be possible to identify groups of genes with related functions or pathways that can be used as therapeutic targets. For example, DNA and histone methylation are important biological processes in myeloma which is characterized by overexpression of MMSET, a histone methyltransferase, in t(4;14) myeloma, and mutations in other methyltransferases, such as EZH2 and MLL3, can also be present. Additionally, histone lysine demethylases such as KDM6A (also known as UTX) can be deleted or mutated in myeloma, 44 making histone methylation a common and attractive target for drug therapy. The discovery of BRAF mutations in 4% of myeloma patients has also brought the possibility of targeted therapy to the forefront of myeloma treatment in the clinic. BRAF is part of the MAP kinase pathway, which is activated by RAS through phosphorylation and results in the subsequent activation of the MEK/MAPK/ERK signaling cascade, resulting in proliferation and survival.[45] The BRAFV600E mutation is present in 50%-60% of all melanomas and results in constitutive activation of BRAF, bypassing the requirement for RAS, activating the MEK/MAPK/ERK cascade, and culminating in cell proliferation and malignant conversion.[46] The drug vemurafenib is a competitive selective inhibitor of BRAFV600E which is approved for use in melanoma and results in relative reduction of 63% in risk for death compared to other treatments.[47] Vemurafenib, therefore, represents a potential targeted therapy for patients harboring a BRAFV600E mutation and clinical trials are underway in myeloma to determine its efficacy.

Intraclonal heterogeneity

Like many malignancies, myeloma cells are not uniform within a patient. A great deal of genetic variation exists within the population of tumor cells, and it is this variation that allows the cancer to persist and diversify. The genetic events within a cancer cell consist of 'driver' and 'passenger' lesions, where drivers confer a selective advantage to the progeny. The acquisition of these lesions allows for the rapid evolution of a clone in a Darwinian fashion.

Selection pressures are exerted on the tumor cells allowing the outgrowth of any favorable trait. These selection pressures may give a growth advantage to a cell, confer a better interaction with the bone marrow microenvironment, or even allow independence from the bone marrow resulting in a plasma cell leukemia or an extramedullary tumor.

Aside from this, mutations gained in subpopulations of cells may confer drug resistance, allowing the eventual repopulation of the tumor in a drug resistant state. Although myeloma is considered to be a clonal disease, due to the presence of one V(D)J rearrangement and a monoclonal secreted immunoglobulin, at a genetic level the cells are far from clonal. IGH translocations and hyperdiploidy are accepted as being primary events in myeloma pathogenesis; however, the rate at which other abnormali-

ties are accrued has been less well studied. Studies utilizing FISH were the first to investigate the relationship of abnormalities within a sample by using probes to a translocation and a copy number abnormality and comparing the frequencies. When comparing a translocation with del(13q) it was found that the majority of cells carry the translocation (as expected given it is a primary event) but the proportion of cells with del(13q) can vary dramatically from patient to patient, but is always lower than the frequency of the translocation.48 It can be inferred from these data that the copy number abnormalities occur subsequent to the translocation. By analyzing the disease at different time points it becomes clear that the frequency of any given abnormality increases through MGUS and SMM towards MM in a population of individuals. This has been shown for del(13q), del(17p) and 1q+where the proportion of myeloma patients with an abnormality increases as the disease progresses.[18,49] However, such an analysis can be even more informative if sequential samples from the same patient are used, particularly when they are taken at different stages of disease (for example SMM and MM). Several papers have been published analyzing such patients by FISH and SNP-based mapping array.[49,50] The overarching theme of these papers is that the frequency of abnormalities increases within a tumor sample as the disease progresses, but they are generally always present at low levels in the preceding stage of disease. For example, in a patient there may be 29% of cells with del (17p) when the patient is diagnosed with high risk-SMM and this may increase to 86% when they present with symptomatic MM.51 The genetic landscape of these tumors gets more interesting as the technologies used get more advanced. Using genome sequencing technologies it is possible to estimate the proportion of cells in a tumor mass with any somatic mutation found. This has been achieved in many cancers,[52,53] including myeloma.[38,54] Taking the RAS pathway mutations as an example, it has been shown that these activating oncogenic driver mutations are not necessarily present in the dominant clone. That is, they can be present only in a subset of the cells in the tumor.[38] This is true for NRAS, KRAS and BRAF mutations, indicating that although they are known oncogenic drivers they are not necessarily present early on in the disease and can be acquired as the tumor evolves.

Using information on the subclonal nature of multiple mutations or copy number abnormalities it is possible to piece together the history of a tumor, determining which genetic events occurred first or occurred together.[52,55] This can also be done at the single cell level using FISH withmultiple probes per cell, or at a nucleotide level using singlecell sorting and genotyping assays.[38,55] These techniques clearly indicate a complex substructure of branched evolution in tumor development. Other studies have focused on the genetic evolution of myeloma following treatment.[54] Analysis of tumor DNA collected at multiple time points during a patient's treatment can illustrate the genetic diversity within a myeloma tumor and the effect that treatment has on the dynamics of the sub-clones present. By studying seven time points from diagnosis, remission, four relapse phases and progression to plasma cell leukemia the different subclones present can be seen using arrays, gaining and losing dominance in the myeloma population as the patient undergoes different treatment regimens. Ultimately, the clone that was dominant as the disease progresses to PCL was barely detectable at diagnosis. Given that myeloma exists as multiple foci of lytic lesions throughout the bone marrow, it remains to be determined how these subpopulations of cells relate to one another, whether they evolve independently, and whether they can be treated as a whole.

Conclusions

Myeloma is a genetically complex malignancy in which translocations involving the IGH locus and hyperdiploidy are primary events. These events are followed by an accrual of additional lesions through MGUS and SMM before transforming to MM. These additional lesions include, but are not limited to, chromosomal gains and losses, somatic mutations and DNA methylation changes. It is clear that there is a subclonal genetic structure within the myeloma cell population where copy number and somatic mutations are gained or lost over time, resulting in a mixed population of cells capable of exploiting any selective advantages laid upon them. This intraclonal heterogeneity may prove to be an extra obstacle in the fight towards curing myeloma, but through using therapies towards key genetic mechanisms it should prove possible to selectively target mutated clones.

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