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Genomic instability in Multiple Myeloma-relevance for Clinical Outcome and Efficacy of Therapy

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ABSTRACT

Genomic instability is a driving force in the natural history of blood cancers including multiple myeloma,an incurable neoplasm of immunoglobulin producing plasma cells that reside in the hematopoietic bone marrow. Long recognized manifestations of genomic instability in myeloma at the cytogenetic level include abnormal chromosome numbers (aneuploidy) caused by trisomy of odd-numbered chromosomes; recurrent oncogene-activating chromosomal translocations that involve immunoglobulin loci;and large-scale amplifications, inversions, and insertions / deletions (indels). Catastrophic genetic rearrangements that either shatter and illegitimately reassemble a single chromosome (chromotripsis) or lead to disordered segmental rearrangements of multiple chromosomes (chromoplexy) also occur. Genomic instability at the nucleotide level results in base substitution mutations and small indels that affect both the coding and non-coding genome. Distinctive signatures of somatic mutations that can be attributed to defects in DNA repair pathways, the DNA damage response or aberrant activity of mutator genes including members of the *APOBEC* family have been identified. Here we review recent findings on genomic stability control in myeloma that are not only relevant for myeloma development and progression, but also underpin disease relapse and acquisition of drug resistance in patients with myeloma.

Keywords: plasma cell malignancy; chromosomal instability; DNA damage response; DNA repair; mutation signatures

GENOMIC INSTABILITY IN MYELOMA

Loss of genomic stability control,leading to large-scale chromosomal aberrations is a widely recognized hallmark of human cancer[1] including hematopoietic malignancy plasma cell myeloma a.k.a. multiple myeloma (MM). Aberrations of this sort include deletions, insertions, inversions and translocations,that can be readily detected using conventional Giemsa banding or spectral karyotyping in tumor cells in metaphase of the mitotic cycle[2–3]. Fluorescence in situ hybridization (FISH) and other molecular cytogenetic methods can be used for interphase cells. Myeloma is a rare, difficult-to-treat and, in the majority of cases, incurable neoplasm of terminally differentiated, immunoglobulin-producing B lymphocytes called plasma cells that reside in the bone marrow. Just as it does in other blood and solid cancers, loss of genomic integrity also results in small-scale aberrations of the myeloma genome. These can be discerned with the assistance of next generation sequencing (NGS) of genomic DNA, including whole-exome sequencing (WES) and whole-genome sequencing (WGS).

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Running Head: CIN in myeloma

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NGS technology-a collection of new methods for DNA sequencing developed in the mid to late 1990s and implemented in commercial DNA sequencers by the turn of the millennium-has tremendously empowered researchers to assess genomic instability in myeloma, look for insights into myeloma development and progression, and consider new approaches to personalized myeloma treatment. In contrast to first–generation technology including Sanger sequencing, NGS technology is cost effective and highly scalable, which allows large portions of the genome, such as the entire protein-encoding exome, to be sequenced at once. High-throughput NGS methods include pyrosequencing, ion semiconductor/torrent sequencing, sequencing by synthesis or ligation, nanopore sequencing, and combinatorial probe anchor synthesis. Regardless ofwhich method is chosen for a given project, strong biocomputational support and a stringent data analysis pipeline are required to produce reliable results [4].

Small-scale aberrations of the myeloma genome include base substitution mutations (point mutations), small insertions and deletions (indels), loss of heterozygosity, and copy number changes that affect individual genes or circumscribed chromosomal domains. Genomicinstability in cancer including myeloma-often referred to as chromosomal instability or CIN-is of great clinical significance because it underpins clonal diversification and adaptation processes that facilitate, to name but two outcomes, increased tumor heterogeneity in the course of tumor progression and acquired drug resistance in response to therapy. CIN determines, in part, the duration and depth of the treatment response in patients with myeloma and, thereby,impacts progression-free and overall survival. The relationship of CIN and survival is reflected in myeloma gene expression signatures that may be used for patient stratification and prognostication [5–6]. Telomere length, another measure of genomic instability, is also associated with survival in myeloma [7].

From a comparative tumor biology point-of–view, CIN is a long-recognized and prominent feature of plasma cell tumors (PCTs) that arise in mouse models of human myeloma and related disorders. This includes the classic model of inflammation–dependent peritoneal plasmacytoma in strain BALB/c mice [3, 8–12] developed by Dr. Michael Potter at the United States National Cancer Institute more than 50 years ago [13–14]. Also included are more recently designed, genetically engineered mouse models (GEMMs) of myeloma; e.g., one that is based on the loss of Rrm2b (ribonucleotide reductase regulatory TP53 inducible subunit M2B) [15], a key enzyme in *de novo* deoxyribonucleotide synthesis important for DNA damage repair. CIN is an active area of preclinical and clinical myeloma research that has not only unearthed an abundance of candidate myeloma progression genes [6] but also holds promise for improved determination of the risk with which the myeloma precursor conditions, monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma (SMM), transition to frank myeloma [16–17]. Given the importance of the bone marrow microenvironment in the natural history of myeloma, it is worth noting that preliminary evidence indicates that genomic instability in myeloma may "spill over" to bystander cells in the tumor microenvironment (TME). One example of this, reviewed in greater depth elsewhere [18], is the induction of genomic instability in bone marrow stromal cells (BMSCs) upon exposure to myeloma cells [19–20]. An intriguing example of the opposite; i.e., induction of genomic instability in myeloma by cells in the TME, is the dendritic cell-mediated activation of AID (activation-induced cytidine deaminase) [21].

Fig. 1 shows that CIN manifests itself at all levels of the myeloma genome, spanning the chromosome and higher-order nuclear structure to individual genes. Presented in the section below is a short summary of forms, phenotypes and biological outcomes of genomic instability in myeloma, followed by a brief discussion of underlying sources and biological pathways. Additional information is available in expert reviews on genomic instability in cancer [24] including myeloma [25] and high-risk myeloma [26].

CYTOGENETIC AND MUTATIONAL LANDSCAPE

From the cytogenetic perspective, recently reviewed by Kumar and Rajkumar [27], MM can broadly be divided into neoplasms that harbor either a hyper-diploid genome due to trisomy that preferentially involves odd chromosomes, or a pseudo-or hypo-diploid genome that contains a balanced (reciprocal) chromosomal translocation that recombines the immunoglobulin heavy-chain locus, *IGH*, at 14q32 with an oncogene on one of several partner chromosomes[28,29]-mainly with–*MMSET* at 4p16 [30,31] or, less frequently, *MAF*, [32,33] *MAFB*[34], *CCND1* [35]and *CCND3* [36] at 16q23, 20q12, 11q13 and 6p21, respectively[37]. That translocation-bearing myeloma karyotypes can be notoriously complex, presenting with the kind of "cytogenetic chaos" that is typically seen in solid but not hematopoietic cancers, has been recognized early on by cytogeneticists [38]. Recently discovered chromothripsis and chromoplexy, are extreme forms of chromosomal

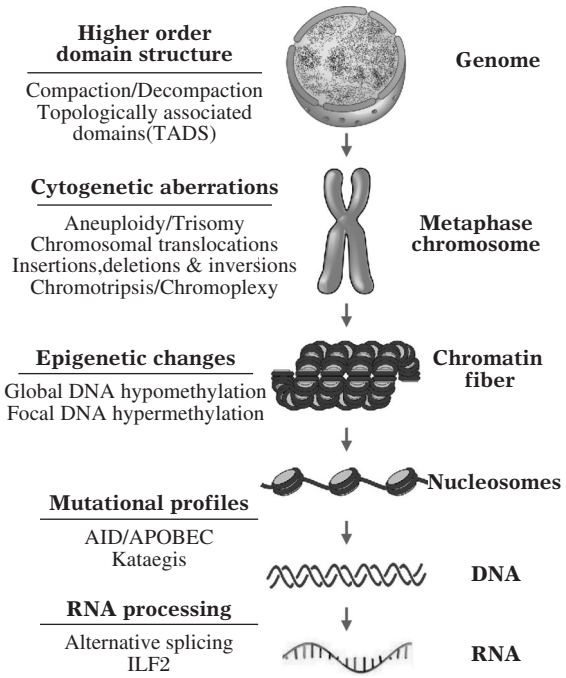

Fig. 1 **Manifestation of genetic instability at all levels of the myeloma genome.** The hierarchical organization of the genome at the chromosomal, chromatin fiber, nucleosomal and nucleotide level is indicated by the labeled scheme. Genomic changes commonly seen in myeloma are listed on the right. Recent findings indicate that myeloma exhibits substantial epigenetic change that relies on a small set of transcription factors, including members of the IRF (interferon regulatory factor), ETS (E26 transformation-specific), MEF2 (myocyte-specific enhancer factor 2), E-Box (enhancer box) andAP–1 (activator protein 1) families of proteins. Also included are E proteins, such as TCF3 (transcription factor 3) a.k.a. E2A (E2A immunoglobulin enhancer-binding factors E12/E47), TCF4 (transcription factor 4) a.k.a. ITF–2 (immunoglobulin transcription factor 2) and TCF12 (transcription factor 12)[22]. Jin et al. also showed that de-compaction of heterochromatin is a defining feature of myeloma cells[99], which is in line with evidence that the myeloma genome undergoes genome-wide DNA hypo-methylation in the course of tumor progression[23]. AID, activation-induced cytosine deaminase; APOBEC: apolipoprotein B mRNA editing enzyme, catalytic polypeptide; ILF2: interleukin enhancer binding factor 2; TADs: topologically associated domains.

breakage and reassembly in myeloma cells that prognosticate poor survival [39,40]. In keeping with the maxim that little if anything in myeloma is fully consistent, tumors carrying the Cyclin D1-activating t(11;14) translocation ($\leqslant$ 20%) tend to have simple karyotypes [25]. What is more, approximately 10% of tumors exhibit no abnormality at all at the cytogenetic level [41]. Myeloma cells also harbor recurrent unbalanced aberrations, most commonly gains at 1q and losses at 1p, 6q, 8p, 13q, 14q, 16q and 17p [41,42]. Gains and losses in these regions are thought to point, respectively, to putative myeloma onco-and suppressor genes-yet the nature of many of these genes remains obscure at this juncture. A newly identified cytogenetic subgroup of myeloma, associated with a highly adverse risk profile features a hyper-haploid karyotype that contains only 30–33 chromosomes[43,44]. This subgroup is typically seen in younger patients and is characterized by both multiple monosomies and loss of p53 function-the latter consequent to monosomy 17 and frequent mutations of *TP53*[45,46].

At the level of individual genes, myeloma exhibits a heterogenous, moderately affected mutational landscape that features a median of 60 somatic mutations detected by WES. In-depth analysis of WES results demonstrated that myeloma cells harbor a number of recurrently mutated genes but lack a consistent hallmark mutation such as the gain-of-function $MYD88^{L265P}$ allele in Waldenström macroglobulinemia[47]. The most commonly mutated genes in myeloma are *KRAS* and *NRAS* (~20% of patients in both cases), followed by *TP53, DIS3*, *FAM46C* and *BRAF* (~10% in all cases) [48]. Additional mutations affecting *TRAF3*, *EGR1*, *SP140*, *FAT3* and a few other genes have been detected, but they are rare and not observed in more than ~5% of patients [49]. Although limited to the exome (2% of the whole genome), the mutational analysis of primary tumor samples has yielded a better understanding of the clonal evolution of myeloma, including difficult questions such as whether mutations that target the same pathway (e.g., *KRAS*–, *NRAS*– or *BRAF*-dependent activation of MAPK signaling) occur in the same cell clone or are distributed among different cell clones admixed in the same diagnostic bone marrow sample[50]. The two possibilities are difficult to distinguish by DNA sequencing. Panel sequencing of the genes mentioned above, which may soon arrive as a commercial assay in clinics[51], will likely facilitate the selection of molecularly targeted drugs, an important step toward individualized myeloma treatment. Panel sequencing may also facilitate the detection of circulating myeloma cells in peripheral blood [52], a promising method that currently relies on genome-wide sequencing of cell-free DNA (cfDNA) [53,54]. Panel sequencing can also be employed asa discovery tool. For example, its clever use recently led to the surprising finding that myeloma cells may harbor kinase-activating fusion genes [55,56] analogous to the *BCR–ABL1* fusion seen in $t(9;22)^{+}$ chronic myeloid leukemia (CML).

MUTATIONAL TARGETS, DRIVERS AND SIGNATURES

Whole-genome sequencing(WGS) provides a

deepinsight into the mutational landscape of myeloma because it covers the vast non-coding portion of the genome(98%) in addition to the protein-encoding portion (2%). WGS revealed that the myeloma genome is littered with many mutations (5×10^3~10×10^3) in both transcribed and non-transcribed regions, with the former including many mutations that target microRNA, small nucleolar RNA and long-noncoding RNA amongst other RNA species[57]. The overwhelming majority of mutations detected by WGS are postulated to represent bystander or passenger mutations; i.e., "genetic noise" or "collateral damage" that results from compromised genomic integrity, but are not relevant for tumor development and progression. Distinguishing the mutational driver from bystander events is a major challenge going forward. A case in point are previously identified "driver" mutations in transcribed genes that were later on found to be barely expressed at the mRNA level[58]. Obviously, the circumstance that mutant alleles may not be translated to mutant protein scastsserious doubt upon the putative tumor-promoting role of the underlying mutations.

By virtue of uncovering distinct mutational signatures in gDNA, WGS has also made a major contribution to the identification of the genotoxic stress that underpins the mutational landscape of myeloma. Four signatures have been identified thus far: (1) methylated cytosine deamination, a generic mutational process observed in many cancers that results in cytosine-to-thymine(C→T) transitions at CpG (guanine) dinucleotide sites; (2) kataegis, a pattern of localized hypermutation that co-localizes with regions of genomic rearrangements and also leads to C→T transitions, but in the context of TpC dinucleotides; (3) APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide), a pathway of somatic mutagenesis that is most frequently found in tumors that harbor MAF or MAFB activating chromosomal translocations, and targets C to undergo transition to T (C→T) or transversion to adenine (A) or G (C→A/G) at TpC sites; (4) AID (activation-induced cytosine deaminase), a mutator enzyme that is essential for V(D)J hypermutation and Ig isotype switching in normal B lymphocytes [59], mechanistically involved in MYC-activating translocations in aberrant B cells [60], and able to mutagenize oncogenes in myeloma (e.g., *CCND1*) that are rearranged by illegitimate trans-chromosomal exchange with the *IGH* locus(e.g., t[11;14] translocation)[61]. The APOBEC signature is of particular interest due to its prognostic impact in myeloma [62].

A recent large-scale WGS analysis of newly diagnosed myeloma (NDMM) by Walker *et al* [63]-supplemented with RNA-seq data and associated with the clinical and outcome results of nearly 1300 patients -greatly expanded the list of putative myeloma oncogenes (*PTPN11*, *PRKD2*, *SF3B1*, *IDH1*, and *IDH2*) and tumor suppressor genes (*UBR5*, *HUWE1*). Interestingly, amongst a total of 63 driver genes, 17 are potentially actionable in terms of pharmacological targeting. Additionally, WGS analysis shed light on myeloma progression pathways that exhibit tumor subtype-dependent preferences, as previously reported by Bolli *et al*.[64]. An interesting emerging theme is transcription-coupled mutagenesis; i.e., mutations in oncogenes that occur solely, or at an increased rate, in tumors in which the expression of these genes is constitutively upregulated by chromosomal translocation. Examples include elevated mutation frequencies in *CCND1* in $t(11;14)^+$ tumors, as mentioned above; *MAF* in $t(14;16)^+$ tumors; and *FGFR3* in $t(4;14)^+$ myelomas. As pointed out in an insightful commentary on the Walker et al. data by Bergsagel and Kuehl [65], the mechanistic basis of other associations revealed by WGS analysis is less clear; e.g., prevalence of gains in 11q, mutations in *FAM46*C and rearrangements of MYC in hyper-diploid tumors. The preferred occurrence of PRDK2 and DIS3 mutations in $t(4;14)^+$ tumors and the association of *BRAF*, DIS3 and ATM mutations in $t(14;20)^+$ tumors also lacks a mechanistic explanation at this time.

The study summarized above and earlier work by Bolli *et al*. [66] have redefined our understanding of genetic drivers of myeloma to include not only mutated driver genes but also chromosome gains and losses, chromosomal translocations, loss of heterozygosity, and the APOBEC mutational signature mechanism[62]. The p53 tumor suppressor, encoded by TP53, is an example of a mutated driver that strongly predicts poor outcome. The short survival of patients with "double-hit" NDMM involving p53[67] and the prognostic value of sub-clonal p53 copy numbers[68] underline the clinical relevance of p53 as a target and contributor to genomic instability in myeloma. Preliminary findings suggest that another tumor suppressor gene, *WWOX*, which is frequently involved in chromosomal translocation [69,70], also falls into the category of driver genes that are able to amplify genomic instability once they have been targeted by somatic mutation.

DEREGULATED DNA DAMAGE RESPONSE

All cells including myeloma are able to deal with a moderate level of genomic damage by activating a network of adaptive changes and biological pathways collectively termed DNA damage response (DDR). The response includes DNA damage recognition,

checkpoint control, cell cycle arrest and, importantly, DNA repair. Depending on biological context, DDR leads to different outcomes; e.g., programmed cell death (apoptosis), which may be followed by immune clearance of apoptotic debris; senescence, which may support a state of tumor dormancy; and survival, the precondition for tumor precursors to continue on the path of neoplastic development and complete malignant transformation. The molecular events that comprise DDR in mammalian cells have been elucidated in detail and grouped into three functional steps: "sensors" that recognize damage, "transducers" that coordinate and effect signal transduction, and "effectors" that execute biological outcomes. DNA repair is comprised of a variety of lesion-specific pathways that include mismatch repair(MMR), base excision repair (BER), nucleotide excision repair(NER), transcription coupled repair (TCR) and DNA double strand break (DSB) repair. The latter employs different molecular machineries and sub-pathways known as homologous recombination (HR), non-homologous end joining (NHEJ), microhomology mediated end joining (MMEJ) and Fanconi anemia (FA) repair. As recently reviewed by others [71], it is abundantly clear that myeloma cells manifest activated, dysfunctional DDR and DNA repair activities (***Fig. 2***) that are involved in tumor development and also important for acquisition of resistance to myeloma drugs, disease relapse, and patient survival.

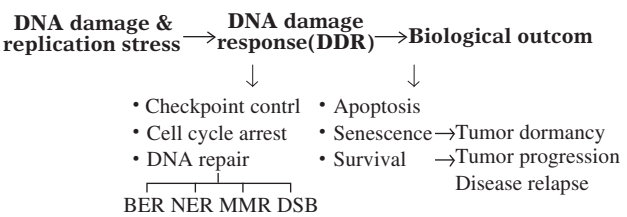

Fig. 2 **DNA damage response in myeloma.**

While inactivation of p53 and loss of ATM or ATR function upstream of p53 are crucial oncogenic events in the natural history of solid tumors, changes of this sort are infrequent in myeloma and thus unlikely to govern the DDR in neoplastic plasma cells. On this backdrop, it is of great significance that Cottini *et al*. recently implicated YAP1(Yes associated protein 1) in DNA damage-dependent apoptosis in myeloma [72,73]. YAP1 is an activator of the Hippo signaling pathway that controls organ size by virtue of regulating cell proliferation and apoptosis, and causes a hippopotamus-like phenotype of tissue overgrowth if hyperactivated by certain mutations. Cottini *et al*. showed that pervasive DNA damage in myeloma cells leads to the activation of a p53-independent pro-apoptotic network that is centered on the nuclear re-localization of ABL1 kinase, which is widely known for its key role in chronic myeloid leukemia (CML) and Philadelphia chromosome-positive (Ph^+) acute lymphoblastic leukemia (ALL) and the development of the first-in-class molecularly targeted drug, imatinib (Gleevec®). Although nuclear ABL1 triggers cell death via interaction with YAP1 in normal cells, low YAP1 levels in myeloma-due to genetic inactivation or reduced expression-prevent nuclear ABL1-induced apoptosis(***Fig. 3, left***). This may be relevant for myeloma treatment, because YAP1 is under the control of serine-threonine kinase, STK4, and pharmacological inactivation of STK4 may restore YAP1 levels and, thereby, kill myeloma cells(***Fig. 3, right***). This provides the rationale for the development of YAP1 activators [74] for patients with myeloma harboring low YAP1 levels. Of interest from the tumor development point-of-view, the above study led to the intriguing hypothesis that inactivation of the ABL1-YAP1 axis may substitute for loss of p53 function in myelomagenesis.

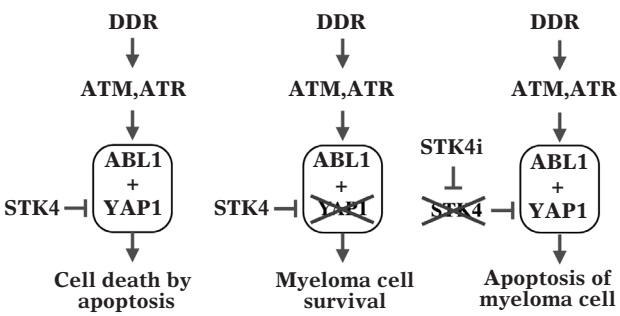

Fig. 3 **Killing myeloma by activating YAP1**. Unlike normal cells in which nuclear ABL1 triggers cell death via interaction with YAP1 (left panel), this pathway is defect in a subset of myeloma that feature low levels of YAP and, thereby, avoid apoptosis (center panel). Since YAP1 is down regulated in myeloma cells by STK4, pharmacological inactivation of the kinase using small-compound inhibitors may restore YAP1 levels to the point at which programmed cell death is triggered (right panel).

The successful development of bortezomib (Velcade) and related next-generation inhibitors, now commonly used as backbone drugs for myeloma treatment, has moved the proteasome to the center stage of myeloma research. Recent findings have linked the DDR in myeloma with the regulation of protein homeostasis via ubiquitination and deubiquitination upstream of the proteasome. Ubiquitination is a sequential enzymatic process that covalently attaches the 76-residue polypeptide ubiquitin to client proteins, thus targeting them for proteasomal degradation or regulating their functional properties such as enzy-

matic activity, subcellular localization and interaction with other proteins. Just like other post-translational modifications, ubiquitination can be reversed by a sizeable family of ($n \leqslant 100$) deubiquitinases (DUBs), which can be classified into six subfamilies based on sequence and domain conservation. DUBs are able to cleave ubiquitin from target proteins, edit ubiquitin chains on proteins, or process ubiquitin precursors in order to maintain a pool of free ubiquitin necessary for normal cell function[75]. Das *et al*. recently demonstrated the involvement of the ubiquitin specific peptidase 1 (USP1) in the myeloma DDR, and showed that a small-drug USP1 inhibitor designated SJB3–019A, decreased the viability of myeloma cells and overcome bortezomib resistance(***Table 1, row 5***). This relied on a mechanism that included the co-inhibition of the Fanconianemia complex and the homologous recombination (HR) sub-pathway of DSB repair[76]. Similar findings were obtained in studies on another DUB known as proteasome regulatory particle lid subunit RPN11(***Table 1, row 3***)[77], for which a candidate small-molecule inhibitor, capzimin, is available as lead compound for further development[78].

Table 1 DNA damage response (DDR) including DNA repair genes implicated in chromosomal instability (CIN) in multiple myeloma

Row	Gene symbol	Gene name	Alias	Pathway	Reference
1	BCL6	BCL6 transcription repressor		DDR	[83]
2	ILF2	Interleukin enhancer binding factor 2		DDR	[98]
3	PSMD14	Proteasome 26S subunit, non-ATPase 14	RPN11	DDR	[75]
4	SIRT6	Sirtuin 6	SIR2L6	DDR	[77]
5	USP1	Ubiquitin specific peptidase 1	UBP	DDR	[74]
6	YAP1	Yes associated protein 1		DDR	[70–72]
7	APEX1	Apurinic/apyrimidinic endodeoxyribonuclease 1	HAP1	BER	[88]
8	APEX2	Apurinic/apyrimidinic endodeoxyribonuclease 2	APE2	BER	[88]
9	ERCC3	ERCC excision repair 3, TFIIH core complex helicase subunit	XPB	NER	[89]
10	MALAT1	Metastasis associated lung adenocarcinoma transcript 1		DSB(NHEJ)	[82]
11	RECQL	RecQ like helicase	RecQ1	DSB(HR)	[78]

DDR: DNA damage response; BER:base excision repair; NER: nucleotide excision repair; DSB:double strand break repair; NHEJ:non–homologous end joining; HR:homologous recombination.

Myeloma´s DDR is also regulated via epigenetic mechanisms, as recently shown by studies on the role of the histone deacetylase, SIRT6 (sirtuin 6), in genomic stability control(***Table 1, row 4***). SIRT6 is a NAD^+ dependent enzyme that is highly expressed in myeloma cells and associated with adverse prognosis. The mechanism by which SIRT6 operates in myeloma depends in part on the downregulation of the mitogen-activated protein kinase (MAPK) pathway. This involves both interaction of SIRT6 with the ETS transcription factor, ELK1, and activation of DNA repair pathways via checkpoint kinase 1 (CHEK1), a serine-threonine kinase that coordinates DNA damage and cell cycle checkpoint responses[79]. Another regulator of genomic stability in myeloma is RecQ like helicase(***Table 1, row 11***), a DNA-unwinding enzyme identified as one of the most downregulated genes in a genome-wide expression screen of myeloma responding to DNA methyltransferase(DNMT) inhibition (DNMTi)[80]. The helicase, encoded by *RECQL*, is significantly overexpressed in myeloma compared to normal plasma cells, and an increased *RECQL* message is associated with poor prognosis in patients with myeloma. Genetic downregulation of *RECQL* induces cell death (apoptosis) and DSBs in myeloma (***Fig 2, right***), while upregulationprotects from melphalan and bortezomib cytotoxicity. Mechanistically, the pharmacologic downregulation of *RECQL* using DNMTi relies on a microRNA called miR-203 (***Fig. 4***).

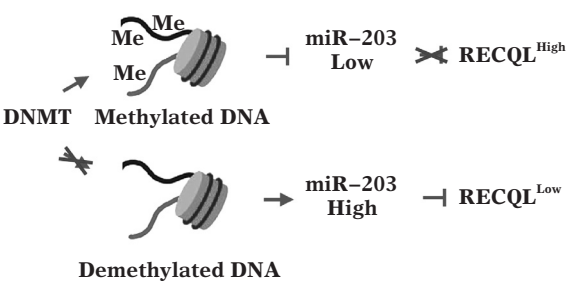

***Fig.4* DNMT inhibition chemo-sensitizes myeloma using a mechanism that involves the down regulation of RecQ like helicase.** Aberrant methylation-dependent repression of miR-203 leads to upregulation of RECQL by diminishing the efficacy with which miR-203 inhibits the expression of the helicase (indicated by red X in upper panel). High levels of helicase in myeloma cells promote resistance to replication-dependent DNA damage and myeloma drugs. Treatment of myeloma cells with DNMTi (red X in lower panel) results in de-repression of miR-203 and downregulation of RECQL, causing loss of resistance to replication stress and myeloma drugs.

The above-mentioned study on RecQ like helicase revealed an interesting parallel to a therapeutic vulnerability of breast, ovarian and other solid tumors that are sensitive to PARP(poly ADP-ribose polymerase) inhibition because they lack functional BRCA1 or BRCA2 tumor suppressor proteins. In myeloma,RecQ interacts with PARP, raising the possibility that DNMTi synergizes with PARPi to kill myeloma cells, in which RecQ is expressed at high levels. However, this has not been demonstrated. Following up on the finding on miR-203, researchers have implicated additional miRs in genomic instability in myeloma. Examples include the discovery of amiR-29b-dependent pathway[81], the finding that miR-137 induces genomic instability in an aurora kinase A (AURKA)-dependent manner[82] and the observation that regulation of DNA ligase III in myeloma involves miR-22[83]. No doubt, the list of miRs is poised to expand as the field moves forward and additional RNA species will betested. A long non-coding RNA(lncRNA) dubbed MALAT1 has also come into play[84] and the master regulator of B-cell development,BCL6, has been shown to down regulate the DDR in myeloma[85].

DEFECTIVE DNA, REPAIR AND RNA PROCESSING

Similar to the CIN score mentioned above, Bernard Klein and his associates devised a DNA repair score that is predictive of progression-free and overall survival of patients with myeloma[86]. The risk score is based on the expression of 22 genes that encode DNA repair proteins in myeloma, with 17 and 5 genes linked to poor and good outcome, respectively. The score´s robustness underlines the impact of aberrant DNA repair in myeloma. Findings that myeloma backbone drugs such as alkylating agents (melphalan) and proteasome inhibitors (bortezomib) affect the capacity of myeloma cells to maintain genomic stability[87] quickly led to the postulate that an enhanced understanding of DNA repair mechanisms in myeloma will lead to new therapeutic approaches based on the concept of synthetic lethality. This arises when a combination of deficiencies in two genes (e.g., gene X and a DNA repair gene) causes cell death, whereas a deficiency in only one of the genes (gene X) does not. The first example of a molecularly targeted drug that successfully exploited the concept of synthetic lethality (first FDA approval in 2014) is the development of PARP inhibitors for the treatment of solid tumors deficient in *BRCA1* and *BRCA2* function. These tumor suppressor genes are important for the error–free HR pathway of DSB repair. Interestingly, Neri *et al*. showed that myeloma cells may be pharmacologically sensitized to PARP inhibition by bortezomib-induced "BRCAness," in which bortezomib-dependent impairment of HR results in synthetic lethality in combination with PARP inhibition[88].

Continuing with studies on HR-dependent DSB repair, several independent groups demonstrated that dysfunctional, elevated HR underlies genomic instability and increases the burden of genetic change that leads to drug resistance and disease progression in myeloma[89,90]. An interesting new development is the finding that the base excision repair (BER)-associated apurinic/apyrimidinic (AP) nucleases, APEX1 and APEX2 (***Table 1, rows 7–8***), contribute in important ways to the regulation of HR in myeloma [90]. Genetic and pharmacological inhibition of APEX1 and APEX2 inhibited HR activity in myeloma cells, using a mechanism that involved the ability of AP nucleases to regulate the expression of RAD51 recombinase. RAD51 depends in part on the *TP73*-encoded tumor protein p73, which is related to p53 and is also considered a tumor suppressor, although debates about its role in malignant development persist. Another recent advance is the implication of NER in CIN in myeloma. Szalat *et al*. showed that expression of the canonical NER gene *ERCC3* (excision repair cross–complementation group 3) significantly impacted the outcome in newly diagnosed MM patients treated with alkylating agents(***Table 1, row9***). The investigators also demonstrated that targeting xerodermapigmentosum complementation group B (XPB), the DNA helicase encoded by *ERCC3*, led to NER inhibition, which in turn significantly increased sensitivity to alkylating agents [91].

There is also some preliminary evidence for mismatch repair (MMR) deficiency in myeloma detected with the help of a high-resolution florescent method of microsatellite instability (MSI) analysis [92]. Following up on earlier observations suggesting the MSI phenotype occurs in~20% of myelomas [93] or as many as~50% of myelomas [94], Miyashita *et al*. recently used the high-resolution florescent MSI assay to unequivocally demonstrate microsatellite instability in 2 of 20 (10%) patients with myeloma-one at the time of diagnosis and the other in the course of disease progression[95]. Although it appears MMR deficiency is not frequent in myeloma, it maybe still be worthwhile to identify patients of this type because experience with solid tumors, particularly colorectal carcinoma,showed that MSI can determine responses to cancer immunotherapy. One striking exampleis long-term remissions in a subset of patients with metastatic disease treated with immune checkpoint inhibitors[96,97].

Post-transcriptional RNA processing adds another

layer of complexity to the maintenance of genomic stability in myeloma[98]. RNA processing includes the concerted modification of the splicing patterns of transcripts involved in DNA repair and maintenance of genomic stability in response to genotoxic stress [99]. The alternative splicing program governed by DDR relies on the proper regulation of the expression, localization and activity of RNA-binding proteins (RBPs) that serve as gatekeepers of genomic integrity[100]. Since the disruption of regulatory interplay between RBPs and DDR may promote genomic instability and the acquisition of drug resistance, the targeting of aberrant RBP function during DDR is an active area of preclinical myeloma research, aimed at developing new approaches to sensitize myeloma cells to DNA damaging agents. The potential to therapeutically target aberrant RBP activities in myeloma has been demonstrated by Marchesini et al. The investigators showed that genomically unstable and aggressive myelomas carrying 1q21 amplification have acquired dependency on 1q21 induced overexpression of RBPILF2 (interleukin enhancer binding factor 2)[101]. ILF2 functions as akey modulator of HR repairin myeloma (***Table 1, row 2***).Mechanistically, high ILF2 expression drives resistance to genotoxic agents by modulating the translocation of YB1 (Y-box binding protein 1) from the cytoplasm to the nucleus where it interacts with a splicing factor that promotes mRNA splicing of transcripts involved in HR repair. These findings are consistent with clinical observations that"1q21 patients" benefit less from high-dose therapy than non-1q21 patients, and that nuclear expression of ILF2 is highly correlated with that of YB1 in 1q21 myeloma. The findings also agree with laboratory results showing that YB1 downregulation following DNA damage leads to γH2AX accumulation and caspase 3 activationin myeloma cells. Importantly, the work by Marchesini *et al*. suggests that ILF2 may serve as a good biomarker of aggressive myeloma, and that blocking the ILF2 signaling axis may enhance the efficacy of myeloma therapies that are based on DNA-damaging agents.

KEY POINTS AND FUTURE DIRECTIONS

Genomic instability in myeloma, which manifests itself at all levels of the genome, drives myeloma development and progression. Cytogenetic changes comprise aneuploidy, chromosomal translocation, amplification and indel, and fragmentation & reassembly of whole chromosomes: chromotripsis and chromoplexy. Changes at the nucleotide level include base substitution mutations and small indels in putative myeloma driver genes. Myeloma mutational signatures have been identified and mechanistically attributed to aberrant DNA damage responses that include dysfunctional DNA repair pathways. An open question is whether genetic predisposition to myelomamay be linked to compromised stability control of the myeloma genome. The molecular mechanisms underlying CIN in myeloma, including the role (if any) of the bone marrow tumor microenvironment,also warrant additional research. In a subsequent review to be published in this journal, we will present new findings on myeloma germline risk, including ethnic and familial factors. We will address research gaps on the mechanism by which germline risk alleles may promote genomic instability in myeloma, including the open question as to whether genetic modifiers of myeloma development act in tumor cells, the tumor microenvironment, or in both. Finally, we will propose new research directions that concentrate on the biological function of myeloma risk and genetic instability alleles, the potential links between the germline genome and somatic changes in myeloma, and the need to elucidate genetic modifiers in the tumor microenvironment.

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