1. BIOLOGY AND PRECLINICAL

TIME-RESOLVED MULTI-LAYERED PROFILING IDENTIFIES RESOLUTION OF RIBOSOME COLLISIONS AND TRANSLATIONAL RECOVERY AS MECHANISMS CONTRIBUTING TO PROTEASOME INHIBITOR RESISTANCE

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Background. Multiple myeloma (MM) is characterised by a high dependence on intracellular protein homeostasis (proteostasis), a vulnerability exploited therapeutically by proteasome inhibitors (PIs) that disrupt protein degradation and induce proteotoxic stress. PIs have significantly improved clinical outcomes, but molecular mechanisms underlying adaptive resistance of MM cells to PI-induced stress remain incompletely understood. Ribosome collisions (RCs) are events that occur during compromised mRNA translation when ribosomes slow or pause, causing trailing ribosomes to physically collide. This triggers translational stress signaling aimed at resolving RCs and restoring homeostatic protein synthesis. Whether proteasome inhibition induces RCs in MM cells and whether RC resolution mechanisms contribute to adaptive PI resistance remains unknown.

Methods. MM cell lines were exposed to a short pulse of carfilzomib (Cfz) to mimic clinical pharmacokinetics and followed by multi-omic analyses. RNA-seq and ribosome profiling (ribo-seq) were performed at 4h (acute stress), 24-48h (early recovery) and 6 days (late recovery) post-treatment. Global protein synthesis was assessed by puromycin incorporation, intracellular amino acid levels were quantified by targeted metabolomics (LC-MS/MS), and changes in gene and protein expression and phosphorylation were analysed by qRT-PCR and immunoblotting.

Results. Cfz rapidly induced RCs, activation of the ZAKalpha-P38 initiated ribotoxic stress response (RSR), reduction of amino acids, activation of the integrated stress response (ISR) and suppression of global protein synthesis. Despite this translational repression, ribo-seq revealed selective enhancement of translation of proteasome subunits and stress-response genes in line with a “proteasome bounce-back” mechanism. During the 24h-48h period, RCs were resolved and both RSR and ISR signalling progressively decreased. Simultaneously, global protein synthesis recovered and even became transiently elevated. Integrated RNA-seq and ribo-seq analyses revealed extensive translatome reprogramming, marked by increased translation of ribosomal subunits and translation factors. In the late recovery phase, fully proliferative MM cells no longer showed evidence of RCs, or RSR or ISR signalling. However, Cfz-resistant MM cells maintained a distinct translational programme characterised by increased translation of the protein synthesis machinery. Pharmacological inhibition of the RSR using a ZAKalpha-P38 inhibitor or suppression of translation with rapamycin or homoharringtonine markedly impaired MM cell recovery from proteasome inhibition, indicating that both acute RSR signalling and the subsequent translational reactivation are required for overcome PI-induced stress.

Conclusions. RCs occur as an early consequence of proteasome inhibition in MM cells and are accompanied by rapid activation of both the ribotoxic and the integrated stress response and a pronounced repression of protein synthesis. Resolution of RCs and reactivation of translation are key determinants of recovery from PI-induced stress and represent potentially targetable vulnerabilities to overcome PI resistance.