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β2-microglobulin – a trigger for NLRP3 inflammasome activation in tumor-associated macrophages promoting multiple myeloma cell progression

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Pro-inflammatory macrophages, as significant constituents of the tumor microenvironment in multiple myeloma (MM), are key promoters of disease progression, bone destruction, and immune-impairment. Consequently, the identification of endogenous mediators of these inflammatory processes open novel therapeutic avenues against major pathological features of MM. We identify beta-2-microglobulin (β2m) as an important driver in the initiation of inflammation in myeloma-associated macrophages (MAMs). Lysosomal accumulation of phagocytosed β2m in patient derived MAMs promoted β2m amyloid aggregation, resulting in lysosomal rupture and ultimately in the production of active interleukin (IL)-1b and IL-18. Interestingly, this process strictly depended on the activation of the NALP3 inflammasome after β2m accumulation. Moreover, depletion or silencing of β2m in MM cells abrogated inflammasome activation in a murine MM model. Finally, the specific disruption of NLRP3 or IL-18 diminished tumor growth and osteolytic bone destruction normally promoted by β2m-induced inflammasome signaling. Taken together our results provide novel mechanistic evidence for β2m's role as an NALP3 inflammasome activator during MM pathogenesis. Moreover, inhibition of NALP3 highlights one potential novel therapeutic approach to combat this severe malignancy. *The authors marked with an asterisk equally contributed to the work.

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The expression of small heat shock protein AllbpA from mycoplasma *Acholeplasma laidlawii* in *E. coli* cells promotes the formation of amyloid structures

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The main function of small heat shock proteins (sHSPs) is the prevention of protein aggregation in the cell. Besides, sHSPs have been shown to be involved in many other processes including the biofilm formation. Thus, in *E. coli*, two sHSPs IbpA and IbpB indirectly influence biofilm formation. When absent, cells are subjected to endogenous oxidative stress and consequent overproduction of indole, that in turn inhibits formation of the biofilm. Here we show that the overproduction of sHSP AllbpA from phytopathogenic mycoplasma *Acholeplasma laidlawii* restores at high temperatures the biofilm formation and increases the amyloid structures content in *E. coli* cells lacking their own IbpB. The full-length AllbpA and proteins with deletions of putative functional terminal motifs (AllbpAΔN12, AllbpAΔC14, AllbpAΔN12C14) were overexpressed in *Escherichia coli* wild-type and strains with deletions of own sHSPs (ΔEcIbpA or ΔEcIbpB). The crystal violet staining of the biofilms revealed that the biofilm formation was restored in ΔEcIbpB cells producing the full-length AllbpA. Furthermore, the Thioflavin S and Congo red staining of the biofilms revealed that the removal of one of sHSP in *E. coli*, with the simultaneous expression of AllbpA, leads to increased formation of amyloid structures. Increased amyloids were also observed during overexpression of AllbpAN12 in the *E. coli* ΔEcIbpA and AllbpAC14 by cells of the *E. coli* ΔEcIbpB, respectively. Amyloids in the matrix were also detected during overexpression of AllbpA with double deletion (AllbpAΔN12C14) in both knockout *E. coli* strains. Thus, the sHSPs can be involved in biofilm formation also via amyloids synthesis, however, the molecular mechanism of regulation requires further study. The work was supported by RFBR grant 20-34-90066.

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Intrinsically disordered regions of alanine: glyoxylate aminotransferase shape its fitness and function

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Intrinsically disordered regions (IDR) play a key role in shaping the plasticity of proteins and often define their function. However, how protein evolution co-opts IDRs to impact on the population of native protein conformers and their individual fitness has remained unexplored. Alanine:glyoxylate aminotransferase (AGT) is a liver pyridoxal 5'-phosphate-dependent enzyme involved in the detoxification of glyoxylate and the cause of primary hyperoxaluria type I (PH1) when dysfunctional. In Caucasian populations, AGT is present in two allelic forms, the major (AGT-Ma) and the minor (AGT-Mi) alleles, the latter increasing the susceptibility of AGT to PH1-causing mutations. By solving the crystal structure of AGT-Mi we identified three distinct regions exposed to the solvent that have a defined structure in AGT-Ma but are disordered in AGT-Mi. Molecular dynamics showed that AGT-Mi samples more flexible conformations than AGT-Ma supporting the hypothesis that IDRs originate from an enhanced plasticity of the entire structure. Characterisation of variants from a library of these three regions shed light on their effect on enzymatic activity and intrinsic stability of AGT. In addition, the analysis of the behaviour of selected hits from the library in human cells, paired with determination of the interactome of AGT-Ma and AGT-Mi, revealed the impact of IDRs on protein fitness and function at a cellular level. This work establishes that naturally occurring conformers generating by taking advantage of subtle instability of a protein can modulate its function and intracellular fitness. *The authors marked with an asterisk equally contributed to the work.