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Author(s)
Eid Mohammed Mansour

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GANP promotes immunoglobulin V-region diversification by regulating the choice among DNA repair pathways

Eid Mohammed Mansour

熊本大学大学院医学教育部博士課程医学専攻免疫学

指導教員

阪口 薫雄 教授
熊本大学大学院医学教育部博士課程医学専攻免疫学
Abstract of the Thesis

Background and Purpose: Maturation of the immune system and the establishment of protective immunity relies on site-directed, transcription-coupled programmed induction of DNA breaks with its subsequent repair in germinal center (GC) B-cells, leading to immunoglobulin diversification and affinity maturation. GANP is a protein selectively upregulated in GC B-cells. The purpose of this study is to elucidate the molecular mechanisms by which GANP would possibly regulate the diversification of the immunoglobulin (Ig) V-region gene.

Methods: To address this issue, we implemented the well-characterized chicken DT40 B-cell system. Using different mutants lacking proteins involved in SHM (AID, Ung) or DNA repair (Rad54, Ku70) and implementing classical tools for measuring gene conversion i.e. sIgM gain and SHM i.e. sIgM loss assays we elucidated the role played by GANP in Ig diversification. We also implemented survival assays using DNA damaging drugs, to assess cell survival upon GANP overexpression or GANP deficiency. Immuno-precipitation and western blots are used to elucidate GANP interaction with DNA repair proteins in Ramos B cells.

Results: GANP Haplo-sufficiency caused reduction in the rates of IgV GCV, and conversely, the introduction of ganp cDNA enhanced the sIgM-gain by GCV in DT40 cells. The results indicated that GANP is an integral molecule in GCV process. Also, GANP increased the SHM rate in the IgV\(_L\) in an AID dependent manner. Using reporter constructs to measure DNA repair activity, GANP clearly promotes homologous recombination repair (HRR), while it suppresses the activity NHEJ repair. Moreover, GANP could efficiently restore compromised GCV/HR in homologous recombination (HR)-defective Rad54\(^{-}\) cells, but suppressed it in Ku70\(^{-}\) mutant cells lacking the non-homologous end-joining (NHEJ) pathway, suggesting that GANP regulates the choice of DNA repair pathways. In cell survival assay, GANP sensitizes the cells to Etoposide, while it protects cells against Camptothecin induced damage, confirming its suppressive effect on NHEJ repair and positive regulation of HR repair. GANP interacts with DNA-PKcs, a core NHEJ protein, in Ramos B cells. This interaction of GANP with DNA-PKcs undergoes dissociation upon Etoposide treatment or AID overexpression, however their association is not altered in AID deficient cells, denoting that this interaction is DNA damage dependent.

Conclusions: GANP protein regulates the choice among DNA repair pathways provoked post AID-induced lesions at the transcriptionally active IgV region, directing the repair to HR repair by abrogating NHEJ repair, thus positively impacting both B-cell survival and IgV functional integrity.