PD-L1 deglycosylation promotes its nuclear translocation and accelerates DNA double-strand-break repair in cancer

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Presenter: Shia-En Chen **Date/Time:** 2025/02/20, 16:10-17:00

Commentator: Prof. Shih-Chieh Lin **Location:** Room 601, Med College Building

Background: Programmed death ligand-1 (PD-L1) is encoded by the *CD274* gene and is a transmembrane glycoprotein primarily localized on the plasma membrane of cancer cells. High PD-L1 expression not only promotes immune evasion in human lung cancer but also contributes to resistance to ionizing radiation (IR) therapy. A previous study demonstrated that IR upregulates PD-L1 expression in cancer and is associated with double-strand breaks (DSBs). However, the underlying mechanism remains unknown. Non-homologous end joining (NHEJ) and homologous recombination (HR) are the two major pathways for DSB repair. Since NHEJ is the primary pathway for repairing IR-induced DSBs, the authors hypothesize that PD-L1 overexpression may be associated with NHEJ-mediated DSB repair, ultimately leading to cancer resistance to IR therapy.

Objective: Investigate the activation mechanism of the DSB repair pathway induced by PD-L1 upregulation.

Results: A genome-scale CRISPR/Cas9 screen in human lung cancer H460 cells identified CD274, the gene encoding PD-L1, as a negatively selected gene, indicating that its loss sensitized cancer cells to IR. Further experiments confirmed that depletion of endogenous PD-L1 not only impaired DSB repair but also reduced cell viability following IR exposure. Time-lapse imaging revealed that PD-L1 translocated from the membrane to the intracellular region after IR exposure. Meanwhile, IR promoted PD-L1 deglycosylation, reducing its molecular weight from 45 kDa to 33 kDa. Treatment with tunicamycin, a glycosylation inhibitor, further enhanced PD-L1 accumulation in the nucleus and cytoplasm. Notably, the glycosylation site N219 was identified as a key determinant of PD-L1's intracellular localization. CKLF-like MARVEL transmembrane domain-containing 6 (CMTM6), known to regulate transmembrane protein transport and interact with PD-L1, was found to be essential for PD-L1 nuclear translocation in response to IR. After IR exposure, PD-L1 depletion significantly reduced NHEJ activity while increasing HR, suggesting that PD-L1 preferentially promotes NHEJ for DSB repair. Co-immunoprecipitation results showed that, upon IR exposure, PD-L1 interacted with Ku80, a key NHEJ factor that recognizes DSBs, through its IgC domain. Both in vitro and in vivo experiments demonstrated that disrupting PD-L1's interaction with Ku80 impaired IR-induced NHEJ repair and increased the sensitivity of lung cancer cells and tumors to IR.

Conclusion: IR exposure promoted PD-L1 deglycosylation and nuclear translocation via CMTM6, facilitating its interaction with Ku80 to accelerate NHEJ repair DSBs.