TARGETING THE NANOG-CRY1 AXIS SENSITIZES IMMUNOTHERAPY-REFRACTORY TUMORS AND ENHANCES EFFICACY OF T CELL-BASED IMMUNOTHERAPY BY REINVIGORATING THE ANTITUMOR IMMUNITY CYCLE

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Background T cell-based immunotherapies have revolutionized the treatment of various cancers. However, only a minority of cancer patients can achieve a durable response because of the emergence of immunotherapy-refractory tumors that block the steps of anti-tumor immunity cycle. Therefore, the identification of clinically available targets that disrupt antitumor immunity is required to develop potential combination therapies. Recent studies reported that epigenetic modifiers including circadian regulators involve in immunotherapy-refractory phenotypes such as resistance to T cell-mediated killing and T cell infiltration. However, the molecular mechanisms that drive immunotherapy-refractory phenotypes are largely unknown.

Methods We integrated analyses of human cancer patients from The Cancer Genome Atlas (TCGA) cohort and preclinical T cell-based immunotherapies-refractory tumor models (Yumm2.1 P3, B16 P3, and CaSkI P3) to identify the cryptochrome 1 (CRY1) as a candidate to overcome the clinical limitations of immunotherapies. The role of CRY1 in resistance to immunotherapy conferred by the NANOG axis was determined by comprehensive biochemical and molecular biology techniques in vitro. Tumor treatment experiments were performed to provide proof-of-concept evidence that CRY1 inhibition is a clinically available strategy to solve unmet clinical needs of T cell-based immunotherapies.

Results The emergence of immune-refractory phenotypes was highly associated CRY1 expression in tumor cells and that CRY1 expression was transcriptionally upregulated by NANOG. Interestingly, in melanoma patients classified as non-responders to anti-PD-1 treatment, circadian rhythm-unrelated function of CRY1 but not circadian rhythm-related was significantly associated with the immunotherapy-refractory phenotypes. Mechanistically, we found that CRY1 contributed to NANOG-driven immunotherapy-refractory phenotypes via formation of complex with HDAC1 to promote its epigenetic silencing of E3 ligases such as APC3, TRIM17. The NANOG-CRY1 axis was conserved in various types of human cancers, and inversely associated with anti-tumor immune response and overall survival of cancer patients. Furthermore, CRY1 inhibition using the small-molecule agent, a potential reliever of tumor immunity cycle is a clinically available strategy to solve unmet clinical needs of T cell-based immunotherapies.

Conclusions Thus, our findings demonstrate that circadian rhythm-unrelated CRY1 potentiates the immunotherapy-refractory phenotypes by interaction with HDAC1 to mediate epigenetic regulation in NANOG+ immunotherapy-refractory tumor cells. Thus, our findings implicate the NANOG/CRY1 axis as a central molecular target for controlling immunotherapy-refractory tumors and provide a rationale for combining CRY1 inhibitors to reverse the refractoriness of tumors to T cell-based immunotherapy.

REFERENCES

Ethics Approval All mice were maintained and handled under the protocol approved by the Korea University Institutional Animal Care and Use Committee (KUIACUC) (KOREA-2021-0049). All animal procedures were performed in accordance with recommendations for the proper use and care of laboratory animals.

Consent The authors have declared that no conflicts of interest exist.