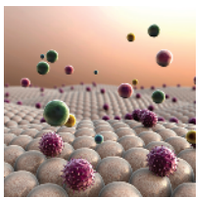
Melanoma

Major finding: Tumor neoantigen–specific CD4+ T-cell reactivity was observed in four of five patients with melanoma.

Approach: Intratumoral CD4+ cells were incubated with autologous B cells loaded with candidate tumor neo-epitopes.

Impact: Strategies to augment CD4+ T-cell responses to neoantigens may be effective in melanoma and other cancers.

INTRATUMORAL CD4+ T CELLS RECOGNIZE MELANOMA NEOANTIGENS



Cancers with high mutational loads, such as melanoma, are considered attractive candidates for immunotherapy because mutations can create tumor-specific neoantigens that are foreign to the immune system. Tumor-infiltrating CD8+ T cells have been shown to recognize neoantigens, but despite the known importance of CD4+ T cells ("helper T cells") in tumor control in preclinical models, it is unclear whether neoantigen-specific CD4+ T-cell reactivity is a common feature of human cancers. Linnemann, van Buuren, Bies, and colleagues developed a screening platform to evaluate CD4+ T-cell responses to tumor neo-epitopes in patients with melanoma in which peptides spanning tumor-specific nonsynonymous mutations identified by whole-exome sequencing were loaded onto immortalized autologous B cells and incubated with intratumoral CD4+ T cells, after which supernatants were evaluated for the presence of cytokines such as IFNγ as a measure of CD4+ T-cell activation. Overall, neoantigen reactivity was observed in 4 out of 5 patients, and against multiple neoantigens in two patients. In all cases, the neoantigen was preferentially or exclusively recognized when compared with the nonmutated parental peptides, and all mutations for which CD4+ T-cell reactivity was detected were unique to that tumor among over 400 melanomas. Two of the patients analyzed experienced clinical responses to adoptive T-cell therapy, and in both patients, neoantigen-specific CD4+ T-cell responses in peripheral blood were induced by therapy. In one case, these neoantigen-reactive CD4+ T cells produced IFNγ when incubated with the tumor cells themselves, providing evidence for direct recognition of neoantigens displayed on tumor cells, at least in some cases. These results indicate that CD4+ T-cell reactivity toward patient-specific neoantigens is a common feature of melanoma and suggest that therapeutic strategies to boost CD4+ T-cell responses may be effective in melanoma and other cancers with similar mutational loads. ■

Linnemann C, van Buuren MM, Bies L, Verdegaal EM, Schotte R, Calis JJ, et al. High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma. Nat Med 2015;21:81–5.

DNA Repair

Major finding: SUMOylation regulates the dosage of activated Fanconi anemia ID complex to promote genome stability.

Mechanism: SUMOylation of FANCI and FANCD2 results in their polyubiquitylation and extraction from DNA lesions.

Impact: SUMO/ubiquitin–dependent regulation may be a general mechanism at replication-associated breaks.

UBIQUITYLATION AND SUMOylation CONTROL DNA DAMAGE REPAIR COMPLEX BALANCE

The Fanconi anemia (FA) pathway, which detects and repairs DNA interstrand crosslinks, is highly regulated by posttranslational modifications. Although FA-associated proteins are functionally modified via phosphorylation and monoubiquitylation, additional mechanisms have also been suggested to regulate this pathway. Gibbs-Seymour and colleagues found that Fanconi anemia, complementation group I (FANCI) and FANCD2, which comprise the ID complex, were SUMOylated in response to DNA damage, which triggered polyubiquitylation and subsequent extraction of the ID complex from sites of DNA damage. ID complex SUMOylation occurred exclusively on chromatin in response to genotoxic stresses, in particular agents that cause replication fork stalling. Deletion studies and *in vitro* SUMOylation assays identified protein inhibitor of activated STAT1 (PIAS1) and PIAS4 as the primary SUMO E3 ligases responsible for this modification. Additionally, both ATR-mediated phosphorylation and monoubiquitylation of the ID complex were required for its SUMOylation, which was antagonized by sentrin-specific peptidase 6 (SENP6). SUMOylation resulted in recruitment of the SUMO-targeted ubiquitin ligase ring finger protein 4 (RNF4), which polyubiquitylated the ID complex with both K48- and K63-linked moieties. Perturbation of RNF4 levels or activity resulted in increased FANCI and FANCD2 levels at sites of DNA damage, indicating that SUMO-dependent polyubiquitylation of the ID complex limits its accumulation at DNA lesions. Consistent with this idea, ubiquitylation of the SUMOylated ID complex promoted its extraction from stalled replication forks by the ubiquitin-dependent DVC1 (also known as SprT-like N-terminal domain 1)–p97 segregase complex. Mutation of SUMOylation sites on FANCI inhibited DVC1–p97-mediated removal of the ID complex and failed to rescue sensitivity to genotoxic stress in FANCI-deficient cells, supporting the hypothesis that initial SUMOylation of FANCI is critical to control ID complex occupancy at sites of DNA damage. Overall, these data indicate that SUMOylation of the ID complex regulates its dosage at DNA lesions to promote genome stability. ■

Gibbs-Seymour I, Oka Y, Rajendra E, Weinert BT, Passmore LA, Patel KJ, et al. Ubiquitin-SUMO Circuitry controls activated Fanconi anemia ID complex dosage in response to DNA damage. Mol Cell 2015;57:150–64.