Immunootherapy

**Major finding:** GITR costimulation induces antitumor immune responses via T₉,9 differentiation and IL9 production.

**Mechanism:** Differentiation of IL9-producing T₉,9 cells is enhanced via the TRAF6–NFκB pathway and IL4 signaling.

**Impact:** GITR-induced IL9 mediates tumor regression by promoting dendritic cell function and CTL responses.

**GITR agonists facilitate antitumor responses via T₉,9 cells and IL9**

Glucocorticoid-induced TNF receptor–related protein (GITR, also known as TNFRSF18) stimulates antitumor responses via T-cell activation in a variety of animal cancer models, but the detailed mechanisms of its action are not well understood. Kim and colleagues found that the GITR-specific agonistic antibody DTA-1 suppressed tumor growth in a syngeneic mouse model of cancer, but not in IL4 receptor–deficient (Il4r−/−) mice. DTA-1-induced tumor regression was mediated by CD4+ T-cell-dependent production of IL9, which was absent in Il4r−/− mice. Furthermore, DTA-1–triggered IL9 expression increased the number and activity of tumor-specific CD8+ CTLs by enhancing the cross-presentation function of tumor-associated dendritic cells (DC) in vivo; these effects were reversed by treatment with a neutralizing antibody to IL9. CD4+ T-cell–driven production of IL9 was stimulated under T₉,9-polarizing conditions in mouse cells treated with DTA-1 and in human cells treated with an agonist antibody to human GITR, demonstrating that GITR costimulation promotes the differentiation of T₉,9 cells. In addition, GITR costimulation shifted the balance of induced regulatory T cells and T₉,9 cells in favor of T₉,9 differentiation both in vitro and in vivo. IL9 production in DTA-1–treated cells was increased even under IL4-abundant conditions, but was reduced by pretreatment with a chemical inhibitor of the NFκB pathway. Consistent with this finding, DTA-1 treatment upregulated expression of TNF receptor–associated factor 6 (TRAF6), a mediator of NFκB activation, and failed to increase IL9 production in TRAF6-deficient CD4+ T cells under T₉,9-polarizing conditions. Taken together, these findings suggest that GITR costimulation promotes T₉,9 cell differentiation and IL9 production via activation of the TRAF6–NFκB pathway and increased IL4 production, enhancing tumor-associated DC function and facilitating antitumor CTL responses. These findings elucidate the cellular and molecular mechanisms underlying the antitumor activity of GITR agonist immunotherapies and support clinical trials of GITR agonists to generate antitumor immune responses.


DNA Repair

**Major finding:** RAD51 T131P–expressing cells display defective ICL repair but normal HR-mediated repair.

**Mechanism:** RAD51 T131P has constitutive ATPase activity and impaired DNA-pairing and strand-exchange functions.

**Impact:** RAD51 protects DNA and maintains genomic integrity during ICL repair independent of its function in HR.

**A clinical RAD51 mutation disrupts DNA interstrand crosslink repair**

The Fanconi anemia (FA) pathway orchestrates DNA interstrand crosslink (ICL) repair, a multistep process that involves DNA incision, translesion synthesis, and homologous recombination (HR). Mutational inactivation of FA genes leads to developmental defects, cancer predisposition, bone marrow failure, and hypersensitivity to crosslinking agents. Recent work suggests that the HR recombinase RAD51 may be recruited to stalled replication forks prior to FA pathway activation. Consistent with this idea, Wang and colleagues identified a heterozygous mutation in RAD51 (c.391A>C) in a patient with FA symptoms, which resulted in an amino acid change (T131P) at a residue required for ATP binding and hydrolysis. Patient-derived RAD51 mutant cells exhibited hypersensitivity to DNA-crosslinking agents, which was reversed upon overexpression of wild-type RAD51 or CRISPR-mediated knockout of the mutant allele, and defective DNA repair resolution despite an activated FA pathway. Surprisingly, although the RAD51 T131P protein was unable to localize to chromatin and delayed the formation of wild-type RAD51 foci, cells expressing RAD51 T131P were HR proficient. Expression of RAD51 T131P augmented the phosphorylation of the single-stranded DNA (ssDNA) binding protein replication protein A in response to ICL-inducing agents; ssDNA accumulation in RAD51 T131P–expressing cells was attributed to increased activity of the resecting nucleases DNA2 and WRN. Moreover, RAD51 T131P was characterized by unregulated DNA-independent ATPase activity and an inability to promote DNA pairing and strand exchange. Co-mixing of different ratios of mutant and wild-type RAD51 in vitro revealed that both the DNA-pairing and strand-exchange activities of wild-type RAD51 were inhibited by mutant RAD51. However, the dominant behavior observed in vitro was seen only when mutant RAD51 was more abundant than the wild-type protein, explaining why patient cells, in which only 20% of RAD51 is mutant, are HR proficient. These findings show the role of RAD51 in protection of DNA during ICL repair independent of its function in HR and highlight an additional clinical subclass of Fanconi anemia.

A Clinical RAD51 Mutation Disrupts DNA Interstrand Crosslink Repair


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