## Genomic Instability

**Major finding:** The FAN1–FANCD2 complex prevents chromosomal instability and tumorigenesis independent of ICL repair.

**Mechanism:** FAN1 binds to ubiquitinated FANCD2 and regulates progression at stalled replication forks.

**Impact:** A *FAN1* mutation affecting replication fork processing predisposes patients to pancreatic cancer.

### FAN1 ACTS AS A TUMOR SUPPRESSOR PREVENTING GENOMIC INSTABILITY

Mutations in Fanconi anemia complementation group D2 (FANCD2) underlie the development of Fanconi anemia by inhibiting the repair of interstrand crosslinks (ICL), which block processes such as DNA transcription and replication. ICL repair requires ubiquitination (Ub) of FANCD2 lysine 561, which is bound by the FANCD1/FANCI-associated nuclease 1 (FAN1). However, the molecular mechanism underlying Ub-FANCD2–mediated ICL repair and the role of FAN1 in the FANCD2 complex remain unknown. Using a mutant FAN1, which cannot bind ubiquitin, and nuclease-defective *Fan1*$^{nd/nd}$ mouse embryonic fibroblasts (MEF), Lachaud and colleagues showed that FAN1 recruitment by Ub-FANCD2 is not required for ICL repair. However, *Fan1*$^{nd/nd}$ MEFs exhibited high levels of chromosomal abnormalities in response to replication fork-stalling agents, suggesting that FAN1 nuclease activity prevents chromosomal abnormalities at stalled replication forks without affecting ICL repair. Consistent with these findings, *Fancd2*-null cells exhibited high levels of chromosomal abnormalities in response to fork-stalling agents, which were not rescued by the non-ubiquitinatable FANCD2 K559R mutant. Double mutant *Fan1*$^{nd/nd}$;*Fancd2*$^{-/-}$ mice had the same level of chromosomal abnormalities in response to fork-stalling agents as single mutants, further supporting the requirement of the FAN1–Ub-FANCD2 complex in preventing chromosomal abnormalities at stalled replication forks. Moreover, DNA fiber analysis indicated that the FAN1–Ub-FANCD2 complex restrains the progression of stalled replication forks. *FAN1* mutations predispose to cancer; 85% of *Fan1*$^{nd/nd}$ mice, but not *Fan1*$^{+/+}$ mice, eventually developed tumors, primarily lymphomas and pulmonary carcinomas, suggesting that FAN1 functions as a tumor suppressor via its nuclease activity. Additionally, a recently identified germline *FAN1*$^{M50R}$ variant associated with predisposition to high-risk pancreatic cancer and required for FAN1 binding to Ub-FANCD2 resulted in unrestrained fork progression and chromosomal instability. Together, these data show that Ub-FANCD2–mediated recruitment of active FAN1 restrains the progression of stalled replication forks, thereby preventing chromosomal abnormalities, and suggest that FAN1 functions as a tumor suppressor that is essential for genome maintenance. ■

*Lachaud C, Moreno A, Marchesi F, Toth R, Blow JJ, Rouse J. Ubiquitinated Fancd2 recruits Fan1 to stalled replication forks to prevent genome instability. Science 2016 Jan 21 [Epub ahead of print].*

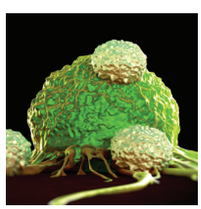
## Immunology

**Major finding:** FOXO1 levels in Treg cells balance antitumor immunity with increased autoimmunity.

**Mechanism:** Activation of AKT and inhibition of FOXO1 expression and localization is essential for aTreg homeostasis.

**Impact:** Depletion of aTreg cells by FOXO1 may promote the antitumor immune response.

### CONSTITUTIVELY ACTIVE FOXO1 IN TREG CELLS INHIBITS TUMOR GROWTH



Regulatory T cells (Treg) expressing forkhead box O1 (FOXO1) mediate the response to self-antigens, preventing autoimmunity. However, excess Treg activity can disrupt antitumor immunity, promoting tumor growth, and tumors with increased Treg infiltration are associated with a poorer prognosis. FOXO1 activity is required for Treg function, and enhances Treg suppression of lymphoproliferative diseases, but its role in activated Treg (aTreg) and resting Treg (rTreg) subsets has not been determined. To address this, Luo and colleagues performed gene expression profiling of aTregs and rTregs. The aTregs preferentially expressed FOXO1-downregulated genes, while the rTregs preferentially expressed FOXO1-upregulated genes. AKT phosphorylation prevents FOXO1 nuclear localization, and in aTregs AKT activation resulted in FOXO1 translocation to the cytoplasm and repression of FOXO1. Conversely, rTreg cells expressed higher levels of FOXO1, which was predominantly localized to the nucleus. *In vivo*, expression of a constitutively active form of FOXO1 (CA) that is insensitive to inhibition by AKT reduced the number of aTreg cells, without affecting rTreg cells, and resulted in CD8$^+$ T cell–mediated inflammation and tissue destruction, indicating that aTregs are essential in suppressing autoimmunity. In a mammary tumor model (PyMT), CA reduced the number of tumor-infiltrating Tregs and suppressed tumor growth. Depletion of CD8$^+$ T cells prevented the tumor suppression, indicating that the antitumor effect is mediated by CD8$^+$ T cells. Altogether, these results suggest that repression of FOXO1 activity is essential for the differentiation of aTregs from rTregs, and excessive FOXO activity promotes loss of aTregs allowing for CD8$^+$ effector T-cell activity. FOXO1 simultaneously inhibits tumor growth and promotes spontaneous autoimmunity, but the high sensitivity of tumor cells to FOXO1-mediated Treg depletion may allow for titration of FOXO1 levels to suppress tumors without inducing autoimmunity. ■

*Luo CT, Liao W, Dadi S, Toure A, Li MO. Graded Foxo1 activity in Treg cells differentiates tumour immunity from spontaneous autoimmunity. Nature 2016;529:532–6.*