Drug Resistance

Major finding: A *BTK* mutation in ibrutinib-resistant patients prevents ibrutinib from binding irreversibly.

Mechanism: Gain-of-function *PLCG2* mutations allow BTK-independent B-cell receptor signaling.

Impact: Understanding ibrutinib resistance mechanisms can guide efforts to treat or prevent resistant disease.

RECURRENT *BTK* AND *PLCG2* MUTATIONS CONFER IBRUTINIB RESISTANCE

The irreversible Bruton tyrosine kinase (BTK) inhibitor ibrutinib has shown significant clinical activity in patients with relapsed and refractory chronic lymphocytic leukemia (CLL), with over half of treated patients experiencing a complete or partial response. Although few ibrutinib-treated patients have relapsed to date, as the number of patients receiving ibrutinib increases it is crucial to characterize ibrutinib resistance mechanisms in order to devise treatment strategies for those who have relapsed and guide development of combination approaches to prevent resistance from developing. Woyach and colleagues performed whole-exome sequencing on peripheral blood samples at baseline and relapse from 6 patients with ibrutinib-resistant CLL. Strikingly, 5 of 6 patients had acquired a C481S mutation in *BTK* affecting the residue to which ibrutinib binds, and 2 patients had distinct mutations in phospholipase C gamma 2 (*PLCG2*), which encodes a kinase that acts immediately downstream of BTK in the B-cell receptor (BCR) signaling pathway. The C481S mutation significantly reduced the affinity of ibrutinib for BTK and prevented its irreversible binding, leading to diminished inhibition of signaling downstream of BTK upon BCR activation *in vitro*. The *PLCG2* mutations also were found to reduce ibrutinib sensitivity *in vitro*, but appeared to have a gain-of-function effect, as they promoted BTK-independent BCR pathway activation in the presence of ibrutinib. These findings reveal a tyrosine kinase inhibitor resistance mechanism that is distinct from other mechanisms involving secondary mutations in the target kinase; instead, ibrutinib resistance can arise from a primary mutation in the target kinase or its immediate downstream target, neither of which are recurrently mutated in CLL. Although additional ibrutinib resistance mechanisms likely remain to be discovered, this characterization of patients who have relapsed on ibrutinib may help initiate strategies to prevent or overcome ibrutinib resistance. ■

Woyach JA, Furman RR, Liu TM, Ozer HG, Zapatka M, Ruppert AS, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhbitor ibrutinib. N Engl J Med 2014;370:2286–94.

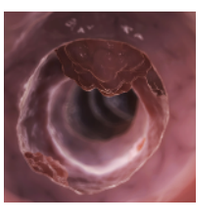
Stem Cells

Major finding: GATA6 depletion decreases adenoma formation and stem-cell expansion via exacerbated BMP signaling.

Mechanism: GATA6 competes with β-catenin/TCF4 for binding to a BMP4 regulatory region to suppress BMP signaling.

Impact: This transcriptional circuit controls tumor initiation by regulating stem-cell self-renewal.

GATA6 MAINTAINS ADENOMA STEM CELLS VIA SUPPRESSION OF BMP SIGNALING



Development of precancerous adenomas through activation of the WNT pathway or inactivation of bone morphogenetic protein (BMP) signaling is the precursor for the development of colorectal cancer. Recent evidence suggests that adenomas harbor a population of stem cells (AdSC) that self-renew and give rise to adenoma lineages similar to normal intestinal stem cells (ISC). Whissell and colleagues discovered a role for the transcription factor GATA6 in regulating AdSCs and suppressing the BMP signaling pathway. Depletion and overexpression of GATA6 in colorectal cancer cell lines confirmed that GATA6 drives expression of the AdSC/ISC marker leucine-rich repeat containing G protein-coupled receptor 5 (LGR5). Conditional knockout of *Gata6* in ISCs resulted in decreased tumor burden and diminished mortality, suggesting that GATA6 promotes colon tumorigenesis. *Gata6*-deficient adenomas harbored a reduced frequency of $LGR5^+$ AdSCs that were restricted to the lowest positions of adenoma glands and displayed upregulation of BMP target genes and nuclear accumulation of phosphorylated SMADs in AdSCs, consistent with the role of BMP signaling in suppressing intestinal tumor formation. Similarly, *Gata6*-deficient adenoma organoid cultures exhibited an increase in BMP pathway components and decreased organoid growth, indicative of impaired AdSC self-renewal. Inhibitors of BMP signaling rescued the proliferative and clonogenic potential of *Gata6*-deficient tumor organoids. Moreover, treatment of mice with pharmacologic BMP inhibitors increased tumor burden and rescued the *Gata6*-deficient phenotype, suggesting a direct role of GATA6 in enhancing adenoma formation through disruption of BMP signaling. In support of this idea, GATA6 competed with β-catenin/T-cell factor 4 (TCF4, also known as TCF7L2) for binding to a distal enhancer region of *BMP4* that contains a SNP linked to colorectal cancer susceptibility, and regulated the expression of many β-catenin/TCF4 targets. Together, these findings demonstrate that GATA6 may play a crucial role in establishing an environment that facilitates colorectal cancer initiation. ■

Whissell G, Montagni E, Martinelli P, Hernando-Momblona X, Sevillano M, Jung P, et al. The transcription factor GATA6 enables self-renewal of colon adenoma stem cells by repressing BMP gene expression. Nat Cell Biol 2014;16:695–707.