The Other Face of ROS: a Driver of Stem Cell Expansion in Colorectal Cancer

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APC mutations causing Wnt activation are commonly found in colorectal cancer, but downstream pathways that facilitate tumorigenesis are unclear. In this issue of *Cell Stem Cell*, Myant et al. (2013) show that Rac1 activation is required for Wnt-driven Lgr5+ intestinal stem cell transformation through ROS production and NF-kB activation.

Inactivation of the adenomatous polyposis coli (APC) gene is the most common mutation (~70%–80%) in colorectal carcinoma (CRC) and is likely the initiating event in colorectal tumorigenesis (Fearon, 2011). Mutated APC is also crucial for the maintenance of CRC, because restoration of wild-type APC expression in CRC cells with inactive APC alleles either reverts the transformed phenotype or promotes apoptosis. APC is a negative regulator of the Wnt pathway, functioning as a self-renewal signal for several different types of adult stem cells (SCs), including intestinal stem cells (ISCs). Notably, inactivation of Wnt signaling in model systems leads to loss of ISCs and tissue integrity, whereas its activation maintains self-renewal (Fearon, 2011). Wnt signals are produced locally, from the niche, to maintain stemness by favoring self-renewal and blocking differentiation (Clevers and Nusse, 2012). β-catenin is a key player in the signaling output of the Wnt cascade. In the presence of inactive APC mutants, as in colon cancer, constitutive activation of β-catenin signaling occurs.

In this issue of *Cell Stem Cell*, Myant and colleagues (Myant et al., 2013) identify Rac1 as another critical component of Wnt activation after APC loss in CRC. Rac1 is a small GTPase that functions within a variety of intracellular pathways to regulate cytoskeletal reorganization, cell migration, proliferation, and survival. Rac1 has been previously implicated in cancer (Krauthammer et al., 2012; Zhou et al., 2013) and in the regulation of the nuclear localization of β-catenin (Wu et al., 2008). Using a number of well-defined mouse models of colon cancer, including mice lacking APC and/or Rac1 and mice expressing mutated Kras, the authors show that Rac1 is activated in CRC, at all stages of tumor development, and that it is indispensable for the manifestation of a critical phenotype of APC loss: increased proliferation of epithelial cells in the intestinal crypts. Notably, Rac1 deletion suppresses proliferation of APC-deficient cells even after experimentally induced activation of Kras, which is also commonly mutated in CRC. Since Kras mutation is among the most frequent mutations associated with progression to colon cancer, Rac1 might be also crucial throughout the colon transformation process. Surprisingly, the authors observed that Rac1 is not required for β-catenin nuclear localization and/or its functional activity in APC absence.

Tumors are heterogeneous populations, consisting of cells with or without tumorigenic properties (respectively termed cancer stem cells [CSCs] and progenitors). Myant et al. show that Rac1 deletion suppresses APC inactivation associated hyperproliferation of both ISCs and progenitors. Accordingly, Rac1 deletion prevents ISC transformation after APC loss. This is particularly relevant for the value of this pathway as a target for pharmaceutical intervention. The targeting of CSCs, in fact, is probably not sufficient for tumor eradication, since it is becoming clear that oncogene expression in progenitors induces SC reprogramming, suggesting that cancer progenitors contribute to maintaining the CSC pool (Visvader and Lindeman, 2012). Further levels of heterogeneity exist in the colon ISCs, where there is evidence for at least two different populations: one highly proliferative and marked by Lgr5, the other relatively quiescent and marked by BMI1 (Barker et al., 2012). The authors show that Rac1 is required for Lgr5+ ISC transformation, but leave the question open for Bmi1+ cells.

They then identify NF-kB as the downstream effector of Rac1 in cells with inactivated APC. Activation of the NK-kB pathway induces a variety of cellular behaviors that are consistent with tumor promoting functions: survival, proliferation, migration, invasion, glycolytic switch, and angiogenesis. Indeed, NF-kB activation has been documented in several tumor types, due to exogenous signals generated by the tumor microenvironment or to somatic mutations of NF-kB pathway components. Because NF-kB is a key regulator of inflammation, it may represent the link between inflammation and tumor development, which is emerging as a critical connection in all steps of colon tumorigenesis (Perkins, 2012). The authors observed increased NF-kB signaling in the APC-inactivated intestinal cells, which was dependent on Rac1 expression. Notably, they demonstrate that constitutive NF-kB signaling partially compensates for Rac1 deletion.

Finally, Myant et al. show that reactive oxygen species (ROS) function as signaling molecules to connect Rac1 and NF-kB. There is an epistatic interaction between Rac1, ROS, and NF-kB in different intracellular pathways. Rac1 is required for NF-kB activation and is part of a protein complex containing NADPH oxidase, which generates superoxide, a major constituent of the pool of intracellular ROS. NF-kB is a redox-sensitive transcription factor that is activated by increased levels of ROS. The authors

show that increased NADPH oxidase activity, ROS intracellular concentration, and NF-kB signaling in the APC-inactivated intestinal cells are all dependent on Rac1 expression. By modulating intracellular ROS levels in vivo through treatment with antioxidants and prooxidants, they further demonstrate that Rac1-driven ROS generation is essential for the effect of APC loss on ISCs and progenitors. In summary, through the elegant use of various mutant mouse models, the authors provide evidence that Rac1-driven ROS production is required for NF-kB activation and initiation of colon tumorigenesis.

Although ROS involvement in tumorigenesis has been known for decades, ROS were initially considered a byproduct of cellular metabolism, contributing to tumor initiation by inducing DNA damage, e.g., as endogenous mutagenic agents. More recently, ROS increase and DNA damage accumulation have been shown to be invariably associated with expression of activated oncogenes in normal cells, a phenomenon that is perceived as integral to tumor development. There is, however, a second face to ROS, distinct from that of damaging agents, which sees them function as signaling molecules in a variety of intracellular pathways (Giorgio et al., 2007). Accordingly, increased ROS might contribute to tumorigenesis by activating specific signaling pathways, as Myant and colleagues suggest here for NF-kB and colon tumorigenesis.

The authors contribute to a further level of understanding of ROS signaling, i.e. the cellular context wherein ROS accumulation becomes critical for transformation. Fine mapping of the identity of tumorigenic intestinal cells reveals that ROS levels in the APC-deficient intestine were particularly high at the crypt base, at cell positions 1–3 (where Lgr5+ ISCs reside), suggesting that deregulated ROS signaling in the proliferating ISCs is critical to colon tumorigenesis. However, it is also clear that, under normal conditions, context-dependent modifications of the redox balance can influence critical SC functions, including proliferation, differentiation, and survival. SCs have smaller mitochondrial DNA copy numbers and higher expression levels of antioxidant genes than their differentiated progeny, which maintain intracellular ROS at low levels. While this might result in an SC-specific mechanism to preserve genome and proteome integrity, it might also allow tolerance to increased ROS levels, thus providing SCs with the capacity to endure the hyperproliferative effect of ROS. Time and technologies seem ripe to investigate the biology of redox changes in SCs.

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Pluripotent Stem Cells from Cloned Human Embryos: Success at Long Last

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Recently in *Cell*, Mitalipov and colleagues report an advance that has eluded scientists for over a decade—the successful derivation of embryonic stem cell lines using somatic cell nuclear transfer, or SCNT (Tachibana et al., 2013).

After the isolation and propagation of human embryonic stem cells (ESCs) was first reported in 1998, many stem cell and reproductive biologists set their sights on being the first to isolate ESCs from SCNT-generated (or "cloned") human embryos. SCNT is a technique where the nucleus of a somatic cell is inserted into a recipient oocyte. Then cleavage of the oocyte containing the donor nucleus can be induced to initiate embryogenesis, which is supported by reprogramming factors in the oocyte. At the time, SCNT was the only potential source of pluripotent cells genetically matched to an