Integrins and Mutant p53 on the Road to Metastasis

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DOI 10.1016/j.cell.2009.12.016

Understanding how tumor cells invade tissues is key to developing drugs to block metastasis. In this issue, Muller et al. (2009) report that a mutant form of the tumor suppressor p53 in cancer cells boosts the endocytic recycling of the adhesion molecule integrin α5β1 and of epidermal growth factor receptor, promoting invasion and metastasis.

Metastasis—the ability of cells in a primary tumor to invade other tissues and to spread throughout the body—is the underlying cause of most cancer deaths. Myriad cellular signaling pathways are known to facilitate tissue invasion and metastasis (Chiang and Massagué, 2008). Many of these pathways are highly cell type and context dependent and so may drive metastasis of only a few tumor types. Hence, pinpointing signaling pathways that facilitate the metastasis of many types of tumors would provide valuable targets for preventing the spread of many different cancers. Inactivation of the tumor suppressor protein p53 through point mutations or enhanced degradation of the protein is a trait common to the majority of human cancers. In this issue of *Cell*, Muller et al. (2009) show both in vitro and in vivo that expression of mutant p53 drives invasion and metastasis through increased recycling of integrins and epidermal growth factor receptor (EGFR) back to the plasma membrane of tumor cells. They further demonstrate that the ability of mutant p53 to drive invasion correlates with its sequestration of p63, a transcription factor and p53 family member.

Given that mutant p53 is frequently overexpressed in many tumors (Selivanova and Wiman, 2007), a key question that has puzzled cancer researchers is why tumors retain this disabled tumor suppressor rather than losing it

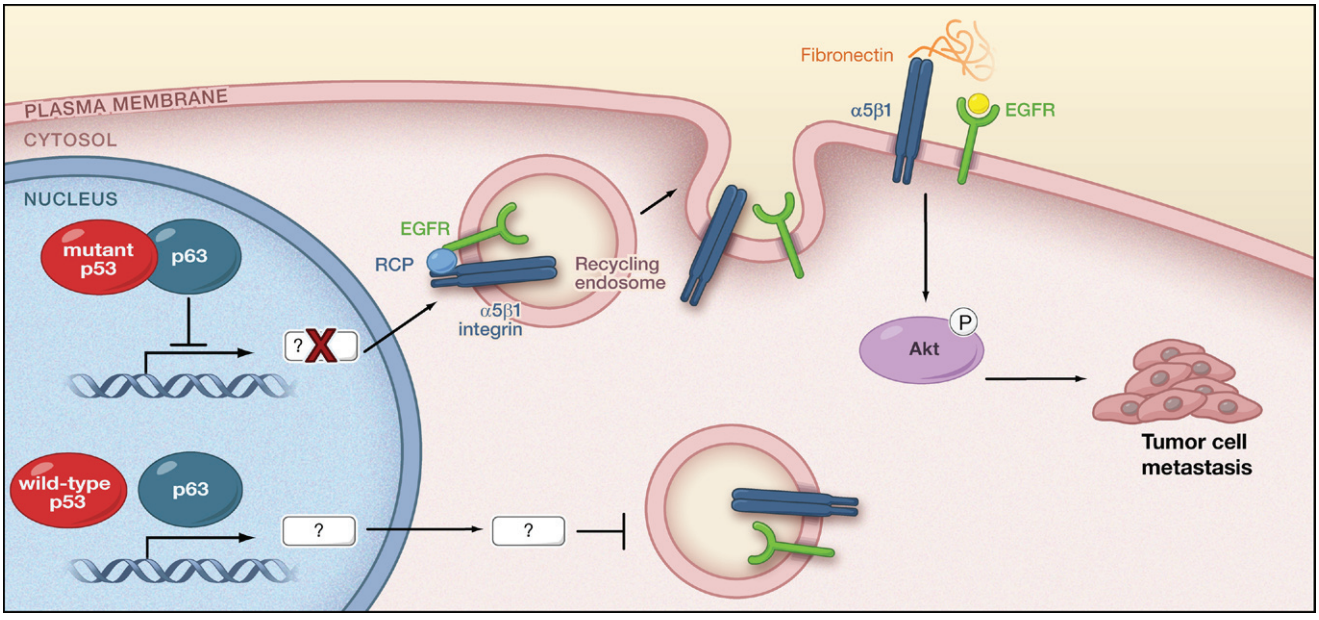

Figure 1. Mutant p53 and Tumor Metastasis
A mutant form of the tumor suppressor protein p53 sequesters p63 (a transcription factor and p53 family member) and blocks its transcriptional activity. This could result in altered expression (red cross) of as yet unknown factors (?) that normally would block the endocytic recycling of the adhesion molecule α5β1-integrin and epidermal growth factor receptor (EGFR). Thus, sequestration of p63 indirectly results in enhanced targeting of α5β1 and EGFR (in a complex with Rab-coupling protein, RCP) back to the plasma membrane. An increase in α5β1-integrin and EGFR at the tumor cell surface boosts the phosphorylation and activation of Akt kinase, which correlates with an increase in the metastatic potential of these cells.

entirely, as is the case with other tumor suppressors such as the retinoblastoma protein or p16. Compelling experimental evidence from mouse models suggests that mutant p53 acquires a gain of function that imbues tumor cells with increased capabilities for invasion and metastasis that are not seen in mice that have lost both *p53* alleles (Brosh and Rotter, 2009). Furthermore, in many types of human tumors, overexpression of mutant p53 is associated with a poor prognosis, supporting the idea of a gain-of-function phenotype for mutant p53, rather than a loss-of-function or dominant-negative effect of wild-type p53 (Brosh and Rotter, 2009). Mutant p53 perturbs the expression of multiple genes through abnormal binding to DNA or to proteins, such as the transcription factors NF-κB and E2F1 and the p53 family members p63 and p73 (Brosh and Rotter, 2009). Sequestration of p63 by mutant p53 appears to play a key role in promoting invasion and metastasis through several mechanisms. Muller et al. (2009) now describe a novel twist in this mutant p53 gain-of-function story with their report of a new link between the intracellular trafficking of key molecules involved in adhesion and invasion (integrins and EGFR) and the inactivation of p63 by mutant p53.

Loss of one *p63* allele weakens the ability of wild-type p53 to carry out its transcriptional function and imbues tumors lacking one wild-type p53 allele ($p53^{+/-}$) with metastatic properties. In contrast, in tumors overexpressing mutant p53 rather than losing expression of wild-type p53, the interaction of mutant p53 with p63 drives the metastatic phenotype (Brosh and Rotter, 2009). Notably, transcriptional profiling has revealed that p63 regulates a whole axis of cell adhesion molecules, including laminin-binding integrins (α3β1, α6β1, α6β4) and a variety of integrin subunits (α5, β1), which bind to fibronectin in the extracellular matrix (Carroll et al., 2006). Recent work has shown that in the presence of mutant p53, p63 and p73 drive expression of β4-integrin resulting in increased invasion capabilities and activation of Akt kinase (Bon et al., 2009). Mutant p53 may switch the p63 transcriptional program over to a tumor-promoting profile by inducing the expression of integrins and increasing their recycling in endosomes back to the cell surface. Muller and colleagues now demonstrate an absolute requirement for α5β1-integrin and EGFR in the invasion ability of tumor cells in vitro. The fact that they did not observe notable changes in the expression of integrin α5β1, EGFR, or the Rab-coupling protein (RCP, which facilitates endocytic recycling) suggests that enhanced endocytic trafficking of these factors (rather than transcriptional control of integrin expression) is the main mechanism for driving tumor invasion in this model.

Muller and coworkers extend these findings and show that mutant p53 facilitates tissue invasion by tumor cells by boosting the interaction between RCP and α5β1-integrin and the endocytic recycling of α5β1 and EGFR. They demonstrate that exogenously introduced and endogenously expressed mutant p53 in several cell lines drives invasion in assays in vitro and confirm this finding in immunocompromised mice transplanted with human tumor cell xenografts. Further, in mice lacking the *Apc* tumor suppressor, expression of mutant p53 increases invasiveness of intestinal tumor cells by 75%. In cell culture assays, enhanced formation of the α5β1-EGFR-RCP complex augments the random motility of tumor cells and their ability to invade fibronectin-containing matrices and correlates with increased phosphorylation of Akt kinase (Figure 1). Consistent with this, the authors demonstrate a correlation between mutant p53 expression and Akt phosphorylation in human tumor samples.

The endocytic trafficking of integrins back to the plasma membrane regulates many adhesion-dependent processes. These include cell motility, cytokinesis, and the interaction between integrins and growth factor receptors that controls the signaling pathways that induce tumor cell proliferation and invasion (Caswell et al., 2009). Importantly, trafficking of different integrin heterodimers is highly interconnected and regulates the rate of recycling of other receptors to the plasma membrane, including EGFR and vascular epidermal growth factor receptor (VEGFR). In fibroblasts, cancer cells, and endothelial cells, the recognition of ligand by αvβ3-integrin and its rapid endocytic recycling suppresses formation and recycling of a complex comprising RCP, α5β1-integrin, and EGFR or VEGFR2 (Caswell et al., 2009). Interestingly, small-molecule inhibitors designed to block αvβ3-integrin during blood vessel formation (angiogenesis) also seem to induce tumor metastasis by boosting the recycling of the α5β1-EGFR-(or VEGFR2)-RCP complex (Caswell et al., 2009).

Recent in vitro and in vivo data challenge the view that αvβ3-integrin is a tumor promoter and instead suggest a potential inhibitory role for αvβ3-integrin in tumor metastasis. Overexpression of αvβ3-integrin in ovarian tumor cells result in a decrease in tissue invasion in vitro, and patients with ovarian carcinoma whose tumors overexpress β3-integrin have a significantly better prognosis (Kaur et al., 2009). These data underscore the clinical relevance of work describing αvβ3-integrin as a major inhibitor of the recycling of the α5β1-EGFR-RCP complex and hence of tumor metastasis. From a therapeutic viewpoint, the new work by Muller et al. together with previous reports raise the possibility that tumors expressing mutant p53 may be eradicated efficiently by blocking the proinvasion gain-of-function effects of mutant p53. This could be achieved either by molecules directly disrupting the interaction of mutant p53 with p63 or by restoring mutant p53 to its wild-type conformation (Selivanova and Wiman, 2007). Yet it is still unclear how increased endocytic recycling of integrins is achieved. Does mutant p53 block p63 transactivation or induce abnormal activity of p63? Which targets of p63 directly regulate the recycling of integrins? Further experiments with different in vitro assays of tumor cells and their interactions with extracellular matrix molecules will hopefully define the critical integrin-mediated pathways that are activated in cells harboring p53 point mutations.

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FANCD2 Hurdles the DNA Interstrand Crosslink

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DOI 10.1016/j.cell.2009.12.006

Left unrepaired, DNA interstrand crosslinks represent impassable hurdles for DNA replication, and their removal is a complicated stepwise process involving a variety of enzymes. In a recent paper in *Science*, Knipscheer et al. (2009) demonstrate that the Fanconi Anemia protein FANCD2 promotes multiple steps of the crosslink repair process.

Like good housekeepers, cells spend considerable energy caring for their genetic material, mopping up DNA damage that may alter the reading of their genetic information. The most difficult type of damage to repair, and at the same time the most detrimental, are DNA interstrand crosslinks (ICLs). ICLs block DNA replication, making their removal an essential requirement for cell survival. Defects in ICL repair underlie Fanconi Anemia (FA), and the severe clinical symptoms of the disorder (blood marrow failure, developmental abnormalities, and cancer predisposition, often leading to an early death) testify to the toxicity of ICLs (Moldovan and D'Andrea, 2009; Patel and Joenje, 2007). Yet, how the proteins in the FA pathway protect against ICLs has long remained a mystery. Using a cell-free system, Knipscheer et al. (2009) now show that FA proteins are directly involved at several steps in the process of ICL repair.

Removal of ICLs mostly occurs in S phase and involves the stepwise involvement of nucleases, specialized DNA polymerases that bypass lesions, and factors that mediate homologous recombination (Moldovan and D'Andrea, 2009). In prior work the authors devised an experimental approach to investigate replication-dependent repair of ICLs using a cell-free replication system of egg extracts from the frog *Xenopus* (Raschle et al., 2008). Their system reveals that replication forks, converging from both directions, initially stop 20–40 nucleotides from the crosslink (Figure 1). One of the forks subsequently moves further and stops again just before the crosslink (at position −1). Nucleolytic incisions on both sides of the crosslink, and DNA polymerization across the lesion, restore one of the chromatids, whereas the other chromatid is most likely repaired through homologous recombination. In an elegant application of their crosslink repair system, Knipscheer et al. now show that loss of proteins in the FA pathway can block both the incision and bypass steps.

Thirteen proteins cooperate in the FA pathway. Eight of these proteins form a ubiquitin ligase complex that monoubiquitinates the substrates FANCD2 and FANCI. In the new study, immunodepletion of FANCD2 from the egg extracts (which also codepletes its heterodimeric partner FANCI) dramatically inhibits crosslink repair, demonstrating that FA is a true DNA repair syndrome. Nucleotide insertion opposite the ICL is blocked in the absence of FANCD2, such that the leading strand progresses only to position −1. Also, the two incisions required to unhook the crosslink are not detected in extracts depleted of FANCD2. All defects can be rescued by adding back the recombinant FANCD2-FANCI complex, but not by adding back a complex containing a point mutant of FANCD2 that cannot be ubiquitinated. Accordingly, when the investigators examine the timing of FANCD2 ubiquitination, they find that this modification occurs precisely when the replication fork reaches the −1 position. FANCD2 ubiquitination, known to be essential for crosslink tolerance, is therefore required to advance the replication fork across the crosslink, by orchestrating the unhooking of the crosslink and DNA synthesis across the lesion.

Bypass of DNA lesions is a potentially mutagenic process performed by specialized polymerases. The FA path-