p53 as an Effector or Inhibitor of Therapy Response

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Although integrity of the p53 signaling pathway in a given tumor was expected to be a critical determinant of response to therapies, most clinical studies failed to link p53 status and treatment outcome. Here, we present two opposite situations: one in which p53 is an essential effector of cure by targeted leukemia therapies and another one in advanced breast cancers in which p53 inactivation is required for the clinical efficacy of dose-dense chemotherapy. If p53 promotes or blocks therapy response, therapies must be tailored on its status in individual tumors.

ACUTE PROMYELOCYTIC LEUKEMIA

Acute promyelocytic leukemia (APL) is the only example of a malignancy cured by targeted therapies (de Thé and Chen 2010; Chen et al. 2011). APL eradication is likely made possible by its genetic simplicity and stability, as well as the availability of very efficient targeted therapies (see below). The disease is driven by the PML/RARA fusion, often associated with activation of FLT3 signaling or Myc trisomy, which promote proliferation and survival in many myeloid malignancies. Expression of PML/RARA in hematopoietic progenitors ex

vivo or in mice drives enhanced self-renewal together with the typical promyelocytic differentiation block (Du et al. 1999; Piazza et al. 2001). Mechanistically, like many fusion proteins, PML/RARA exerts a double dominant negative effect on the pathways controlled by each of its constitutive moieties. RARA is a transcriptional regulator responsive to retinoic acid (RA). PML/RARA represses RARA targets. Moreover, through its ability to bind highly degenerate response elements, PML/RARA also represses signaling by multiple nuclear receptors (Kamashev et al. 2004; Martens et al. 2010). PML/RARA also interferes with PML

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function. PML assembles into nuclear domains that concentrate a variety of enzymes and otherwise unrelated proteins to enhance their post-translational modifications (Lallemand-Breitenbach and de Thé 2010). These PML nuclear bodies (NBs) are stress-responsive structures and PML is required for response to a wide variety of stresses (Wang et al. 1998; Deltre and Bazett-Jones 2004). Specifically, PML is a redox-sensitive protein, which behaves as a reactive oxygen species (ROS) sensor (Jeanne et al. 2010; Zhang et al. 2010). PML oxidation elicits the polymerization of PML onto the nuclear matrix to form NBs (Sahin et al. 2014). This promotes the recruitment and SUMO-conjugation of partner proteins, yielding their transient sequestration within NBs, allowing other post-translational modifications (ubiquitination, acetylation, phosphorylation, etc.) to take place. Thus, on oxidative stress, PML confers an immediate change in the posttranslational modifications of proteins associated with NBs.

PML/RARA expression deorganizes PML NBs (Daniel et al. 1993; Dyck et al. 1994; Koken et al. 1994). PML modulates p53 activity, particularly in response to oncogenic stress (see below). In APL, p53 mutations are very rare and PML/RARA expression was shown to blunt p53 activation, notably for senescence induction (Trecca et al. 1994; Insinga et al. 2004). This supported the idea that NB-disruption antagonizes p53 signaling and thus contributes to APL pathogenesis (Fig. 1). PML NBs also modulate E2F signaling (Vernier and Ferbeyre 2014). Thus, although the first models of oncogenic transformation by PML/RARA stressed the importance of the differentiation arrest through transcriptional silencing, recent studies have highlighted the central role of PML/RARA-mediated disruption of NBs and resulting proliferation boost (Occhionorelli et al. 2011; Gaillard et al. 2015).

Targeting PML/RARA Function and Stability

APL is clinically responsive to RA and arsenic trioxide. RA triggers terminal differentiation of APL blasts and transient clearance of the leukemia. Arsenic cures up to 70% of patients as single agent therapy, while the RA/arsenic com-

Figure 1. Expression of oncogenic RAS elicits PML nuclear body (NB) formation that subsequently triggers p53-mediated senescence. In contrast, PML/RARA prevents oncogenic stress-induced senescence by sequestering PML and disrupting NBs, thus obliterating p53 response.

bination definitively cures virtually all patients in the absence of any chemotherapy (Lallemand-Breitenbach and de Thé 2013; Lo-Coco et al. 2013; Zhu and Huang 2014; de Thé 2015). Initial models aimed at understanding the action of RA all stressed the key role of transcriptional reactivation of PML/RARA in therapeutic response (Licht 2006). RA switches PML/RARA from a constitutive repressor to a hormone-dependent transcription factor, remodeling the chromatin landscape toward a more open state and subsequently releasing the differentiation block (Licht 2009). Yet, the demonstration of the clinical efficacy of arsenic, and the unexpected observation that it also induces APL differentiation *in vivo*, but does not affect RA signaling, raised intriguing questions as to the basis for therapeutic response (Ablain and de Thé 2011).

The unexpected finding that both RA and arsenic target the stability of PML/RARA, by precipitating its degradation by the proteasome, provided a possible unifying mechanism for the activity of these two agents (Zhu et al. 1997, 2001). Indeed, PML/RARA degradation is the only shared biochemical property between

these two otherwise unrelated agents. Studies performed in mouse models of APL have conclusively shown that PML/RARA loss is responsible for the abrogation of APL self-renewal (Nasr et al. 2008; Ablain et al. 2013, 2014). Subsequent studies showed that PML/RARA loss is actually sufficient for differentiation, thus explaining in vivo myeloid maturation on arsenic exposure (Vitaliano-Prunier et al. 2014).

Mechanistic studies aimed at understanding the biochemical basis for therapy-induced PML/RARA degradation revealed that RARA behaves as a RA-inducible degron (Zhu et al. 1999). This likely reflects a universal feedback mechanism wherein nuclear receptors undergo a postactivation degradation through a direct contact with 19S proteasome. In contrast, arsenic targets PML (Zhu et al. 1997). Arsenic directly binds PML or PML/RARA and also indirectly induces their oxidation (Jeanne et al. 2010). This precipitates the formation of PML NBs and, through recruitment of the UBC9 SUMO-E2 ligase, favors the SUMOylation of PML and its associated proteins (Lallemand-Breitenbach et al. 2001; Sahin et al. 2014). HyperSUMOylation of PML triggers recruitment of the SUMO-dependent ubiquitin ligase RNF4, PML, or PML/RARA polyubiquitination and proteasome-mediated degradation (Lallemand-Breitenbach et al. 2008; Tatham et al. 2008). Thus, in addition to changes in gene expression, therapy-induced PML/RARA loss restores the normal organization and function of PML NBs.

p53, a Key Effector of APL Therapies

Mouse models have played a critical role in deciphering the actual basis of APL eradication by the RA/arsenic combination. In particular, they allowed the detailed analysis of the early molecular and cellular events that occur on initiation of RA or arsenic therapy. The first major contribution of mouse models was to show that RA-induced differentiation could be uncoupled from long-term disease clearance. This was a major surprise in the field, as it profoundly questioned the concept of differentiation therapy (Ablain and de Thé 2011). This actually explained why RA and arsenic antagonize each

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other for differentiation (Shao et al. 1998), but sharply synergize for APL eradication in vivo (Lallemand-Breitenbach et al. 1999; Rego et al. 2000). The second essential step was the realization that differentiation is linked to transcriptional activation (or derepression), although loss of self-renewal is the direct consequence of PML/RARA loss (Nasr et al. 2008; Ablain et al. 2013). Subsequent studies indeed revealed that therapy-induced PML/RARA degradation triggers a p53 response. The latter is absolutely required for APL clearance, but not differentiation (Ablain et al. 2014; Bourdeau and Ferbeyre 2014).

The Unexpected Role of Normal PML in Therapy Response

Pioneering studies in the early 2000s showed that PML is an upstream activator of p53 signaling (Ferbeyre et al. 2000; Guo et al. 2000; Pearson et al. 2000). Although multiple studies have confirmed the essential role of PML as a p53 co-activator, how this is actually performed mechanistically remains ill-understood. A variety of mechanisms were proposed, including recruitment of the acetyltransferase CBP or sequestration of HDM2 (Bernardi et al. 2004). In fact, p53 and virtually all of its modifying enzymes may be located within PML NBs. Current views hold that PML forms a scaffold that recruits most p53 modifiers, hence integrating their afferent signals to finely tune p53 signaling, both in its nature and strength (Ivanschitz 2015). In the APL model, PML controls a specific subset of p53 targets implicated in senescence (Ablain et al. 2014). Indeed, PML is not only one of the most specific markers of senescent cells, it is also required for senescence induction in multiple systems (Vernier et al. 2011). Moreover, when overexpressed, a single PML splice variant controls p53 signaling and enforces senescence in primary cells, most likely through recruitment of p14ARF (Bischof et al. 2002; Ivanschitz et al. 2015). Finally, PML is transcriptionally induced on p53 activation, pointing to a feedforward, self-amplifying mechanism (de Stanchina et al. 2004).

Because PML NBs regulate p53 output, one could imagine that their reformation on

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PML/RARA degradation would be implicated in p53 activation. Indeed, PML/RARA-targeting agents reverse the block in p53 function imposed by the oncoprotein and activate a PML/p53-dependent checkpoint with features of senescence (Fig. 2) (Ablain et al. 2014; Bourdeau and Ferbeyre 2014). Importantly, deletion of PML in APL cells abrogates therapy-induced loss of self-renewal and impedes activation of p53 targets. This model should therefore allow mechanistic explorations on the molecular basis for PML control over p53 activity. In particular, it is well suited to investigate whether specific PML-dependent p53 posttranslational modifications are required for the activation of this subset of target genes driving loss of self-renewal.

Arsenic is an extremely potent anti-APL drug, which targets both PML/RARA and PML. Although PML/RARA degradation is evidently beneficial to the disease, the unexpected demonstration of a key role of PML in conveying RA-response raised the tantalizing possibility that arsenic might also act on the normal PML protein to restore/enhance p53 signaling. The curative RA/arsenic treatment was long believed to reflect the more efficient degradation of PML/RARA by two independent proteolysis pathways (Ablain et al. 2011). Yet, arsenic-enhanced NB reformation through binding to the

normal PML protein before its ultimate degradation could also participate in the striking synergy between the two agents. This proposal was strongly supported by the discovery of a mutation of normal PML in a therapy-resistant patient (Lehmann-Che et al. 2014). Remarkably, this mutation (A216V) (Jeanne et al. 2010), immediately adjacent to the arsenic-binding site, is the same as the one observed within PML/RARA in arsenic-resistant patients (Goto et al. 2011; Zhu et al. 2014). Finally, p53 mutations have been reported in some therapy-resistant patients, notably the only ones from which cell lines could be derived (Lanotte et al. 1991; Karnan et al. 2006; Akagi et al. 2009). This may reflect intrinsic resistance of these p53 mutant primary APL cells to oxidative stress triggered by ex vivo culture, which otherwise would induce senescence and prevent long-term propagation.

These findings are important in at least three respects. First, they establish the first model in which p53 is directly implicated in cure by targeted therapies. In that respect, p53 activation also participates in response to Gleevec in animal models of chronic myelogenous leukemias (Wendel et al. 2006). Second, they likely explain why arsenic is so potent as a single agent, through its ability to initiate PML/RARA deg-

Figure 2. Arsenic treatment of acute promyelocytic leukemia (APL) induces PML/RARA degradation by direct binding and oxidation, followed by SUMOylation and RNF4-dependent polyubiquitination. Retinoic acid (RA) targets the RARA part of PML/RARA and initiates its proteasome-dependent degradation. Loss of PML-RARA passive reformation of PML nuclear bodies (NBs). The latter is also enhanced by arsenic through its direct binding onto normal PML. Acute NB reformation triggers p53 activation, presumably via p53-modifying enzymes that reside in NBs, which initiates the senescence of APL cells and results in definitive disease elimination.

Figure 3. High-dose DNA-damaging chemotherapy showed significant clinical benefit in mutant p53 compared with wild-type p53 breast cancer patients. In cells with active p53 signaling, DNA damage induces senescence of cancer cells, which protects tumor-propagating cells from eradication. In contrast, in cells with nonfunctional p53, dose-dense chemotherapy triggers mitotic catastrophe and eliminates tumor-propagating cells, yielding long-lasting complete remissions.

radation and at the same time enforce PML nuclear body reformation by also acting on the normal PML allele. To our knowledge, this is the first example of a drug synergistically acting for on both an oncogene and tumor suppressor, which may explain its extraordinary clinical potency (Chen et al. 2011; de Thé et al. 2012). Third, the unambiguous involvement of normal PML in p53 activation in the context of RA or arsenic responses raises hopes that this checkpoint may be amendable to therapeutic interventions even when the PML gene is not rearranged.

Pharmacological harnessing of PML is possible, because expression of the gene is tightly regulated by interferons (Stadler et al. 1995). Moreover, NB aggregation is regulated by arsenic or other oxidative stresses (Zhu et al. 1997; Lallemand-Breitenbach et al. 2001; Sahin et al. 2014). Enforcing PML expression can facilitate SUMO-initiated proteolysis (Guo et al. 2014; Sahin et al. 2014; Dassouki et al. 2015). Interferon-induced senescence seems to require PML and is enforced by p53 (Chiantore et al. 2012; Fu et al. 2015). It is thus possible that interferon-triggered PML/p53-mediated senescence may become a broad anticancer strategy, particularly in the context of minimal residual disease. In that respect, interferon α has yielded some clinical benefits in the pre-Gleevec periods of chronic myeloid leukemia (Preudhomme et al. 2010; Malagola et al. 2014). Similarly, the combination of interferon and arsenic, which enforces maximal NB formation and partner re-

cruitment (Quignon et al. 1998), exerts clinical activity in HTLV-I associated adult T-cell leukemia (Kchour et al. 2009; Bazarbachi et al. 2011). This likely reflects the degradation of the HTLV-I tax transactivator, an initiator, and a likely driver of ATL by a PML/SUMO/RNF4-dependent mechanism (El-Sabban et al. 2000; El Hajj et al. 2010; Dassouki et al. 2015). Clinical efficacy of this combination may also involve activation of a PML/p53 senescence checkpoint. The striking similitude between this model and APL suggests that the miracle of an APL cure by RA and arsenic may be transposed to some other conditions (de Thé 2015).

p53 STATUS AND DNA-DAMAGING THERAPIES IN BREAST CANCERS

Since the discovery that p53 was a key gene controlling thymocyte apoptosis in response to radiations (Lowe et al. 1993), a very large number of studies have addressed the link between tumor p53 status and response to DNA-damaging therapies. Taken the very high frequency of p53 inactivation in cancers (Kandoth et al. 2013) and the key role of this protein in DNA-damage response, tight connections between the p53 status of any given tumor, and clinical response to DNA-damaging therapies were expected. Yet, most studies have failed to establish any link. When addressing this key translational issue, one has to take a scientific perspective, integrating the following issues: what are the genetics of

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the disease, in particular how heterogeneous is it? What is exactly the molecular effect of the treatment administered? What is the clinical end-point considered (e.g., reduction in tumor size, partial or complete remission, tumor-free survival, overall survival)? How is p53 status determined (e.g., sequencing of mutations, assessment of protein level, functional assays, activation of downstream pathways)? When p53 has undergone a mutation, what is its functional consequence, as all mutations are not identical? Answering all of these questions is essential and significantly more complex than one may imagine at first glance.

A descriptive retrospective clinical study performed in our institution has unexpectedly unraveled a strict dependence of chemotherapy response on loss of p53 transcriptional activity, as determined using the functional yeast assay (Flaman et al. 1995). This specific assay is clearly more informative than p53 detection methods based on immunohistochemistry or first-generation DNA sequencing, although it usually fails to detect *TP53* mutations with mRNA destabilization. Unexpectedly, in advanced breast cancers treated frontline with a dose-intense regimen containing the topoisomerase II inhibitor anthracycline and the alkylator cyclophosphamide, complete pathological responses were only observed in p53 mutant tumors (Bertheau et al. 2002, 2007). This suggested that loss of p53-dependent transcriptional control was essential to the rapid tumor clearance. When trying to further refine the subgroup of responsive patients, it appeared that p53-mutant, estrogen-receptor-negative tumors were the most responsive to this regimen, with complete pathological responses reaching 70% in this subgroup (Lehmann-Che et al. 2010). Another study found that response to cisplatin is associated to loss of *BRCA1* or *TP53* nonsense or frame-shift mutations (Silver et al. 2010), supporting the idea that this group of tumor is exquisitely sensitive to DNA alkylation and strand cross-linking.

One should again stress the importance of the clinical end points. Immediate tumor regression is a very discriminative one, much less complex than survival. In our cohort of patients,

complete remission was the best predictor for cure (Giacchetti et al. 2014). Dose-intense regimens are more likely to be p53 dependent than those involving standard doses (Lehmann-Che et al. 2010). These studies suggest that, as in myeloid leukemias (Fernandez et al. 2009), anthracyclin or cyclophosphamide dose intensification may be very beneficial in estrogen-negative, triple-negative, p53 mutant, or BRCA-1-like breast cancers, which are highly overlapping molecular subgroups (Silver et al. 2010; Giacchetti et al. 2014; Vollebergh et al. 2014; Schouten et al. 2015).

From a mechanistic point of view, these human studies were strongly backed by animal data, initially from xenograft and subsequently from genetically defined mouse models (Varna et al. 2009; Jackson et al. 2012). Collectively, they support a model wherein absence of p53-enforced G₂/M checkpoint in p53-mutant tumor cells treated with chemotherapy allows cross-linked chromosomes to enter mitosis, precipitating mitotic catastrophe (Fig. 3). In cells with proficient p53 signaling, DNA-damaging therapy induces transient growth arrest and senescence, followed by rapid tumor regrowth (Varna et al. 2009; Jackson et al. 2012). Thus, p53 may hamper chemotherapy response in rapidly cycling cells.

TOWARD AN INTEGRATED MODEL FOR p53 IN THERAPY RESPONSE

Thanks to a better molecular characterization of human tumors and the availability of animal models, we are progressively glimpsing the molecular events that take place during cancer therapy. The picture is still blurred, but is progressively clearing. The two examples outlined above stress how varied the situation can be. More than ever, p53 appears to be a central actor in therapy outcome, a true “double-edged sword,” depending on the tumor type and treatment administered (Bunz et al. 1999; Vogelstein and Kinzler 2001). Together with the high proliferation rate, intact p53 signaling is believed to play a role in the exquisite chemosensitivity of testicular seminomas (Riou et al. 1995). Multiple studies have similarly outlined the critical importance of p53 alterations in therapy resistance of chron-

ic lymphocytic leukemia, myelodysplastic syndromes or acute myeloid leukemia (Zenz et al. 2010; Gonzalez et al. 2011; Bally et al. 2014).

We do not wish to infer from these examples that the solution is simple. Many translational studies suffer from confounding factors, including treatment and tumor heterogeneity and ambiguous determination of p53 function. Moreover, multiple ex vivo and in vivo models have rightfully stressed the complexity of the links between p53 status and therapy response. We would only like to highlight the fact that some clinical studies have observed that therapy response was tightly linked to p53 signaling, as expected from genetically clean and therapeutically simple mouse models. Years to come may see p53 status emerge as a key factor for therapeutic decisions. The most frequently altered gene in cancer may, in the end, emerge as the Achilles's heel of tumor cells, provided we find the right arrows.

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