Strategies for p53 Reactivation in Human Sarcoma

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Emerging strategies in cancer therapeutics link the genomic mutational and proteomic landscape, allowing intelligent reasoning on target selection. In this issue of *Cancer Cell*, Piccinin and colleagues use this approach to demonstrate that the mesenchymal protein Twist1 inhibits p53, providing a novel target for reactivation of p53 in human sarcoma.

One of the fundamental aims in biomedical research is the discovery of protein-protein interactions. This has led to an information explosion on biological pathway function, protein-networks, and signaling hubs. In the case of p53, this knowledge is beginning to be translated into cancer patient stratification that complements the advances already being made using gene mutation status and transcription profiling to predict therapeutic responses (Burington et al., 2011). Over 30 years of research has led to over 400 interacting proteins described for p53 that have driven exciting advances in drug development programs that target p53 inhibitors, like ubiquitin ligases (MDM2), histone de-acetylases (SIRT1 and SIRT2), and protein kinases (Aurora, CK1, and CDK; Figure 1). One of the key aims of translational biomedicine is to link this vast information on p53 interactors into clinically relevant cancer therapeutics. In this issue of *Cancer Cell*, Piccinin et al. (2012) bridge this gap and demonstrate that the mesenchymal signaling protein Twist1 forms a clinically relevant inhibitory p53 interaction in human sarcoma.

Our understanding of the genetics of human sarcoma has been facilitated by improved molecular classification using next generation sequencing technologies (Barretina et al., 2010), which has impacted clinically relevant target choice and in rational use of small molecules targeting kinases through to monoclonal antibodies targeting receptors for treating sarcoma (Figure 1). In the case of the most well studied p53 inhibitor of all, MDM2, next generation technologies have shown that *MDM2* is one of the key genes often amplified in human sarcoma (Taylor et al., 2011). Emerging translational data also demonstrate that melanomas overproducing the MDM2 homolog MDM4 form clinical settings in which to apply targeted therapeutics (Gembarska et al., 2012). In human sarcoma, the ratio of spliced MDM4 full-length and smaller isoforms better defined patients with poor survival rates than the mutation status of p53 (Lenos et al., 2012). This affirmation of MDM2 or MDM4 activation in human sarcoma provides a compelling clinical setting to investigate the potential targeting of these relatively well-characterized p53 pathway inhibitors. Current compounds targeting MDM2 protein range from lead molecules interacting with its hydrophobic peptide binding pocket (Vassilev et al., 2004) to the use of conformationally constrained peptides (e.g., stapled peptides), which offers enormous potential to develop a new generation of protein-protein interaction inhibitors to be evaluated clinically (Crunkhorn, 2011). Therefore, there is a significant potential to stratify patients in sarcoma clinical trials that might benefit from MDM2 or MDM4 targeted therapeutics.

Despite this enormous promise in targeting MDM2 to reactivate p53, human sarcomas are a very heterogeneous group of cancers arising from mesenchymal tissues within fat, muscle, peripheral nerves, fibrous tissue, or bone, and the wt-p53 alleles are not always retained. Even in sarcomas with wt-p53 alleles, MDM2 is not always the dominantly expressed p53 suppressor, and identification of such clinically relevant p53-inhibitors is a prime goal. Piccinin et al. (2012) identify such a dominant pro-oncogenic inhibitory protein interaction between Twist1 and the C terminus of p53. Targeting the Twist1-p53 complex not only provides a novel, clinically relevant approach for reactivating p53, but it also complements the existing potential to target synergistically other p53 inhibitory pathways. This knowledge will hopefully give momentum to strategically choose approaches to reactivate p53 in sarcomas by targeting these oncogenic protein-protein interaction networks (Figure 1).

Our limited knowledge of oncogenic protein-protein interactions driving sarcoma, such as Twist1-p53 or MDM2-p53, contrasts with our understanding using genomics that has divided this cancer into two types. Group I sarcomas have near-diploid karyotypes but harbor specific chromosomal translocations, most of which encode chimeric transcription fusion proteins. For example, the PAX3-FOXO1 chimera in alveolar rhabdomyosarcoma reveals how mesenchymal developmental pathways can be exploited by evolving cancers. There are over a dozen other chimeric transcription factors that reveal the genetic diversity of these sarcomas groups. Group II sarcomas are characterized by unbalanced karyotypes reflecting genome instability and mutations in p53 that result in genomic heterogeneity across tumor types. This heterogeneity is highlighted by recent whole-genome sequencing showing that some osteosarcomas and chordomas evolve through a mechanism called chromothripsis (Stephens et al., 2011), reflecting evolution from a single "catastrophic"

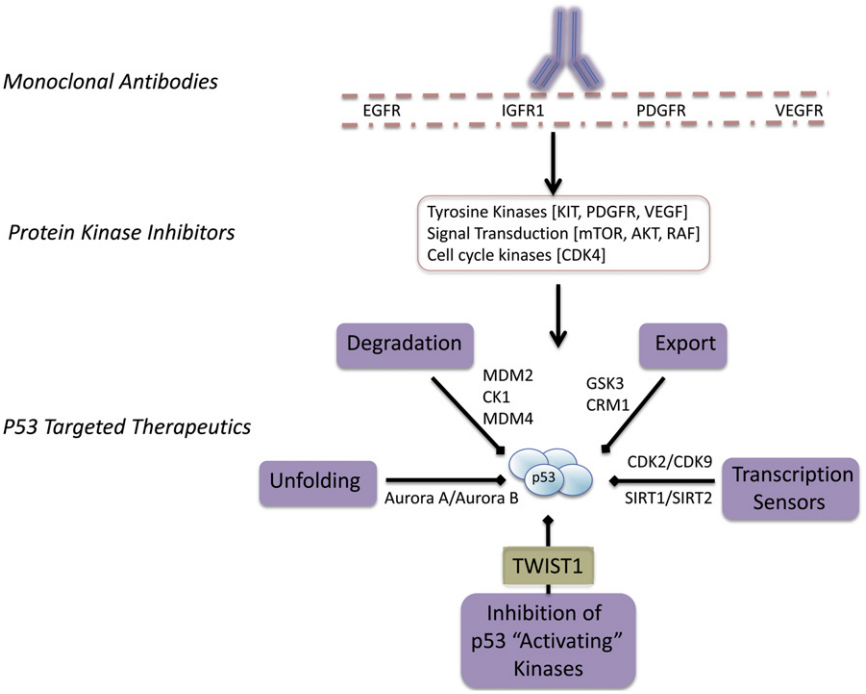

Figure 1. Therapeutic Targets in Human Sarcoma
In human sarcoma, some of the receptor-mediated regulatory pathways that have been identified as therapeutic targets include VEGFR, IGF-R1, PDGFR, and EGFR, which form the focus for monoclonal antibody-based approaches. Similarly, enzymes such as receptor tyrosine kinase growth factors or *CDK4* gene amplification highlight the potential for targeted kinase inhibitors. A subset of human sarcomas expressing wild-type p53 form clusters that might be targeted with p53-activating therapeutic molecules. The p53-inhibitory pathways whose components currently have small molecule drug leads include: (1) degradation pathways comprising MDM2, with its cofactors CK1α and MDM4; (2) unfolding and destabilization of the p53 core domain by the mitotic Aurora kinase family; (3) export pathways comprising the kinase GSK3 or the nuclear receptor CRM1; (4) transcription sensing pathways composed of kinases that regulate Pol-II transcription like CDK2/CDK9 and chromatin remodelling factors like the Sirtuins; and (5) p53 "activation" inhibitors epitomized by Twist1 that block a specific p53 activation phosphorylation. Exploiting the Twist1-p53 interface forms a novel approach for stimulating wt-p53 in certain mesenchymal cancers.

genomic instability event primarily on one chromosome.

In order to discover clinically relevant oncogenic drivers in human sarcoma, Piccinin et al. (2012) reasoned that Group I sarcomas containing the near-diploid karyotype often retain wt-p53 alleles, suggestive of oncogenic pathways that suppress p53 and bypass p53 gene mutation as a selective advantage in cancer development. In order to identify the physiological factors that might attenuate wt-p53 in sarcoma, one likely source would be developmentally programmed pathways, which are intrinsic to mesenchymal tissue, such as Twist1. The Twist1 bHLH transcription factor is involved in tissue speciation following mesoderm induction specifically during embryogenesis and plays a role in metastatic signaling through induction of EMT, and Twist1 signaling was previously implicated in silencing ARF-mediated oncogene activation of p53. The authors thus examined the mechanism whereby Twist1 might attenuate wt-p53 functions in mesenchymal-derived cancers. The authors show that Twist1 binds to the intrinsically disordered motif in the C-terminal regulatory domain of p53, a region that contains the majority of binding sites for p53-interacting proteins as well as covalent modification sites including phosphorylation, ubiquitin-like modification, and acetylation. Twist1 binding specifically attenuates an activating phosphorylation at Ser392 (CK2/FACT phosphorylation motif) implicated normally in stimulating DNA-damage activated gene expression (Bruins et al., 2008). This hypophosphorylated p53 becomes sensitized to degradation by an MDM2-dependent pathway, although the contribution of the other known p53 ubiquitin ligases, such as PirH2 or TRIM28, cannot be ruled out.

The Twist1 interaction with p53 provides another example of the now hundreds of p53 protein-protein interactions. One of the main difficulties in exploiting this vast knowledge of p53 for improving therapeutic strategies to treat cancer is that the clinical penetrance of most of these protein-protein interactions has not yet been defined. A key insight developed by Piccinin et al. (2012) at the outset was to define clinical settings where this inhibitory Twist1-p53 interaction might be clinically important to increase probability of having a future impact for patients. Over 140 human sarcomas of a large range of subtypes were screened for Twist1 expression, and over 60% exhibited strong nuclear protein expression, which in a fraction of cancers can be explained by a copy number gain. Liposarcoma and leiomyosarcoma were the types with highest percentages of Twist1 overexpression. Further, in leiomyosarcomas, there was an inverse correlation between Twist1 protein expression and p53 gene mutation. These data together suggest that there are at least two distinct pathways that drive the evolution of these sarcomas with either mutant p53 or wt-p53 and that high or low expression of Twist1 might play a role in driving these diverse evolutionary paths.

Genomics and proteomics platforms are now allowing annotation of clinically dominant pro-oncogenic driver mutations, gene expression profiling, protein-protein interactions, and potential drug targets with clinical relevance. Identifying clinical niches for such drug targets facilitates the a priori stratification of patients that might benefit from a specific therapy. However, the heterogeneity in human cancer is vast, highlighted by the diversity of sarcoma subtypes. To achieve such rational targeted therapeutics will require highly focused and integrated interdisciplinary collaborative research networks that drive patient stratification using molecular pathology, target choice, drug development, pharmaceutical engagement, and clinical trials. Some of the therapeutic possibilities in human sarcoma include using the currently available lead molecules targeting protein

kinases such as CDK4, monoclonal antibodies targeting receptors like IGF-R1 or EGFR, and peptide-mimetic MDM2 protein-interaction inhibitors that can rescue wt-p53 functions (Figure 1). We can now include the clinically relevant p53 protein interaction from Twist1 as a key target to evaluate for its therapeutic potential. There is enormous promise in drugging such protein-protein interactions, as this forms an untapped and large landscape for drug development (Crunkhorn, 2011).

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IDH1 Mutations Disrupt Blood, Brain, and Barriers

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The first two murine models of IDH1(R132H) mutation provide mechanistic insights into transformation. In hematopoietic cells, inhibition of TET2 and histone demethylases leads to epigenetic alterations and accumulation of hematopoietic precursors. In the central nervous system, inhibition of collagen and prolyl hydroxylases lead to altered microenvironment and defective angiogenesis.

Large-scale sequencing efforts have identified novel genes that are somatically mutated in cancer. Two of the more unexpected genes that have been implicated as recurrent mutational targets are *IDH1* and *IDH2*, which encode isocitrate dehydrogenase-1 and isocitrate dehydrogenase-2, respectively. These enzymes catalyze the conversion of isocitrate to α-ketoglutarate (αKG) in an $NADP^+$ dependent manner. Mutations in *IDH1* were first identified in colorectal cancer, and later, mutations in *IDH1*/*IDH2* were identified in brain tumors, with >70% incidence in secondary gliomas (Yan et al., 2009). Through whole-genome sequencing of a case of acute myeloid leukemia (AML), an *IDH1* mutation was identified, and *IDH1*/*IDH2* mutations were subsequently found in 12%–18% of AML cases (Mardis et al., 2009). Mutations have also been identified in thyroid cancers, chondrosarcomas, and cholangiocarcinomas.

At first it was puzzling how basic metabolic enzymes could be linked to cancer. The mutant IDH1/IDH2 enzymes have decreased enzymatic activity known at that time. However, the observations that the mutations always presented as heterozygous and at highly conserved arginine residues are more consistent with these being gain-of-function. This led to the critical finding that these mutants acquired neomorphic activity converting αKG to 2-hydroxyglutarate (2HG) (Dang et al., 2009; Ward et al., 2010). Tumor samples harboring these mutations had 2HG at levels up to ~100-fold greater than controls. Besides being an intermediate in the Krebs cycle, αKG is involved in other biochemical processes, including synthesis of glutamate, transamination of amino acids, generation of NADPH, and acting as a cofactor for dioxygenase enzymes. The structural similarity between 2HG and αKG suggested that other enzymatic processes may be competitively inhibited by elevated 2HG levels (Xu et al., 2011) (Figure 1).

Through analysis of global DNA methylation profiles in glioblastomas (GBMs), a distinct profile termed CpG island methylator phenotype with elevated genomic methylation was found to be closely associated with *IDH1* mutations (Noushmehr et al., 2010). Subsequently, it was discovered that *IDH1*/*IDH2* mutations were mutually exclusive with *TET2* mutations, a gene encoding an αKG-dependent enzyme involved in DNA demethylation, suggesting that these proteins are involved in the same pathway. Biologic significance was demonstrated through