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Reviving the guardian of the genome: Small molecule activators of p53

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ABSTRACT

The tumor suppressor p53 is one of the most important proteins for protection of genomic stability and cancer prevention. Cancers often inactivate it by either mutating its gene or disabling its function. Thus, activating p53 becomes an attractive approach for the development of molecule-based anti-cancer therapy. The past decade and half have witnessed tremendous progress in this area. This essay offers readers with a grand review on this progress with updated information about small molecule activators of p53 either still at bench work or in clinical trials.

Contents

1. Introduction

For many decades, cancer therapy had relied entirely on chemotherapeutic agents in the forms of antimetabolites, alkylating agents, various alkaloids, cytotoxic antibiotics, and other chemicals that target rapidly proliferating cells through different mechanisms such as topoisomerase inhibition, DNA intercalation, and microtubule polymerization (Chabner & Roberts, 2005). With no other options available, chemotherapy, as a consequence of its inherent cytotoxic nature, went hand in hand with a myriad of side effects, ranging from hair loss and fatigue, to gastrointestinal distress and anemia, and to myelosuppression, immunosuppression, neuropathy, secondary leukemia, and organ failure. The advent of innovative molecular and genetic techniques revolutionized our understanding of the mechanisms that govern cancer and provided the tools to target newfound signaling pathways (Grever, Schepartz, & Chabner, 1992; Sawyers, 2004). Rational drug design and targeted therapy prompted a new era of scientific and clinical inquiry and brought with them the possibility of cancer "magic bullets" that could explicitly engage tumor cells and elicit clinical response without the adverse effects that plague conventional chemotherapy (Amato, 1993; Schnipper & Strom, 2001).

Technological advances such as combinatorial chemistry, high-throughput screening, and chemical genetics made possible the development of small molecules that target specific oncogenic pathways (Nero, Morton, Holien, Wielens, & Parker, 2014). Whereas targeted monoclonal antibodies, which are large globular structures, are generally limited to attaching to antigens expressed on cell surfaces or on secreted factors, small molecules are capable of passing through the lipid bilayers of the cell and nuclear membranes, essentially conceding all receptor, cellular, and nuclear proteins as potential targets (Carter, 2006; Dancey & Sausville, 2003; Huang, Armstrong, Benavente, Chinnaiyan, & Harari, 2004). Perhaps the greatest success story of targeted therapy to date is that of the small molecule tyrosine kinase

Abbreviations: AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; INZ, inauhzin.

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inhibitor imatinib, which has proven to be exceptionally effective against chronic myelogenous leukemia (CML), a white blood cell cancer that previously carried a 4–6 year median survival rate (Druker, 2008). Imatinib targets BCR-ABL1, the fusion gene byproduct of the pathophysiologic chromosomal translocation characteristic of CML and has been largely responsible for the improvement of CML's prognosis to a 90% 5-year survival rate (Druker et al., 2006). Imatinib has also been shown to have activity against two other tyrosine kinases (c-KIT and PDGF-R) and has accordingly been approved for gastrointestinal stromal tumors (GISTs) that are driven by these aberrant kinases, highlighting the value of a small molecule with multiple targets that can be germane to different cancer profiles as well as less susceptible to resistance (Baselga, 2006; Huang et al., 2004; Zitvogel, Rusakiewicz, Routy, Ayyoub, & Kroemer, 2016). Eager to replicate this success, other small molecules were designed against various cancer-associated targets, but the clinical outcomes of many of these drugs were less than desired. Scientists and clinicians alike learned that designing "magic bullet" therapies could not be achieved by simply uncovering and targeting driver mutations, as cancers possess a remarkable ability to develop resistance through a variety of mechanisms (Gottesman, Fojo, & Bates, 2002; Longley & Johnston, 2005). A mutation in the targeted gene affecting drug binding affinity or the upregulation of an alternative signaling pathway can quickly curtail a drug's effectiveness (Glickman & Sawyers, 2012; Redmond, Papafili, Lawler, & Van Schaeybroeck, 2015). These difficulties have led researchers to explore different approaches towards targeted therapy. Rather than focusing on oncogenes upregulated in specific cancers and instead placing more emphasis on genes found to be more frequently and globally mutated in cancers, perhaps therapies can be developed that have a more universal reach and can therefore affect a larger patient population. One such target and the focus of this review is the tumor suppressor p53.

2. p53: the guardian of the genome

The tumor suppressor p53 is the most recognized and researched protein in the study of human cancers. Since its discovery in 1979 (Lane & Crawford, 1979; Linzer & Levine, 1979), more than 80,000 articles have been published about p53 in nearly all disciplines of biomedical research, encompassing cellular and molecular biology, biochemistry, genetics, biophysics, pharmacology, immunology, clinical research, and more. p53's exceptional ability to protect the cell against a wide range of stressful stimuli, such as oxidative stress, nutrient deprivation, hypoxia, DNA damage, telomere attrition, oncogene expression, and ribosomal dysfunction through both transcription-dependent and independent mechanisms (Meek, 2015; Zhang & Lu, 2009; Zhou, Liao, Liao, & Lu, 2012) is matched by its equally impressive array of protective capabilities, including induction of cell cycle arrest, apoptosis, cellular senescence, DNA repair, and inhibition of angiogenesis and metastasis (Bieging & Attardi, 2012; Levine & Oren, 2009; Sengupta & Harris, 2005). Regulation of the cell cycle by p53 transpires at both the G1 and G2 checkpoints through upregulation of CDK-Rb-E2F modulator p21, and cyclin B-CDC2 modulators 14-3-3σ and GADD45, respectively (Laptenko & Prives, 2006; Taylor & Stark, 2001). Apoptosis is achieved through transcriptional activation of subsets of apoptotic genes, of which is dependent on the source and duration of offending stressors (Vousden & Prives, 2009). Mitochondrial-dependent apoptosis ensues following upregulation of mitochondrial proteins, such as PUMA, BAX, and NOXA, while death-receptor-dependent apoptosis is initiated by membrane proteins KILLER/DR5 and Fas/CD95 (Riley, Sontag, Chen, & Levine, 2008; Roos, Baumgartner, & Kaina, 2004; Wu et al., 1997). In addition, activation of autophagy regulator DRAM1 may also play a role in apoptosis (Crighton et al., 2006). p53 can also function outside of the nucleus to directly inhibit antiapoptotic proteins Bcl-2 and Bcl-XL, an example of p53 transcription-independent activity (Green & Kroemer, 2009; Murphy, Leu, & George, 2004). p53's indispensable role in maintaining genome-wide stability has led biologists to christen it the "guardian of the genome". The gravity of p53's importance in preventing tumor formation and progression is perhaps best highlighted by the frequency with which its functionality is abrogated in cancers, as over 50% of tumors either harbor mutations in its gene, TP53, or cultivate posttranslational modifications that abolish its activity (Leroy et al., 2013; Toledo & Wahl, 2006).

The p53 protein spans 393 amino acids and possesses structural domains characteristic of transcription factors, such as a transactivating domain and a DNA-binding domain, as well as an oligomerization domain, a proline-rich domain, and a basic regulatory region (Bell, Klein, Muller, Hansen, & Buchner, 2002; Wang et al., 1993). The N-terminal transactivating domain (residues 1–62) interacts with basal transcription factors and regulatory proteins, including its primary negative regulatory MDM2 (discussed below) and co-activators acetyltransferases p300 and CBP (Kaustov et al., 2006; Meng, Franklin, Dong, & Zhang, 2014; Teufel, Freund, Bycroft, & Fersht, 2007; Wu, Bayle, Olson, & Levine, 1993). Also in the N-terminal region lies the proline-rich domain (residues 63–94), which contains five SH3-domain binding motifs of PxxP sequence and plays a role in p53-induced apoptosis in response to DNA damage (Baptiste, Friedlander, Chen, & Prives, 2002). The oligomerization domain is located in the C-terminal region (residues 325–356) and allows four monomers of p53 to form a homotetramer, a conformation necessary for p53 transcriptional activity (Kitayner et al., 2006; Nagaich et al., 1999). At the end of the C-terminal region is the basic regulatory region (residues 357–393), thought to regulate p53 sequence-specific binding (Friedler, Veprintsev, Freund, von Glos, & Fersht, 2005; Luo et al., 2004). Finally, the core or central DNA-binding domain (residues 94–292) facilitates binding to sequence-specific double-stranded DNA and contains a DNA binding surface comprised of a central β-sheet and two large loops (L2 and L3), stabilized by a zinc ion (Cho, Gorina, Jeffrey, & Pavletich, 1994; Meplan, Richard, & Hainaut, 2000). Stability of the p53 protein is governed by the central domain, and evidence suggests that p53 has evolved to be only marginally stable at physiologic temperatures, as p53 has a melting temperature of 44 °C and a half-life of only 9 min, shorter than its paralogs p63 and p73 (Bullock et al., 1997; Canadillas et al., 2006). p53 is also kinetically unstable, as the central domain cycles between folded, unfolded, and aggregate states at 37 °C (Friedler, Veprintsev, Hansson, & Fersht, 2003). The presence of a zinc ion coordinated by three key cysteine residues and one histidine residue (C-176, C-238, C242, H178) is vital for both stability and accurate binding to DNA-consensus sequences (Butler & Loh, 2003).

Although p53 has indispensible cellular functions, its unintended activation has deleterious effects on normal and developing tissues. Therefore, under physiologic conditions, p53 has a relatively short half-life and its expression is maintained at low levels. The principle regulator of p53 is the oncoprotein and E3 ubiquitin ligase MDM2, also known as HMD2 for its human analog (Haupt, Maya, Kazaz, & Oren, 1997; Honda, Tanaka, & Yasuda, 1997). MDM2 negatively regulates both p53 stability and activity. The N-terminal domain of MDM2 can directly bind to the N-terminal transactivating domain of p53, promoting p53's translocation from the nucleus to the cytoplasm, suppressing its capacity to interact with transcriptional machinery and blocking p53 transcription of target promoters, while the C-terminal RING-finger domain of MDM2, containing E3 ligase activity, ubiquitinates specific lysine residues on p53's C-terminal end, thereby targeting p53 for proteasomal-mediated degradation (Haupt et al., 1997; Kubbutat, Jones, & Vousden, 1997; Marine & Lozano, 2010). MDMX, also known as MDM4 (HDMX/HDM4 for its human analog), is structurally homologous to MDM2 and can both directly bind to and inhibit p53 as well as stabilize MDM2 to potentiate MDM2's ubiquitination capabilities (Gu et al., 2002; Marine et al., 2006; Shvarts et al., 1996). Classically, activation of p53 occurs when interaction between p53 and MDM2/MDMX is perturbed, with different stressors having different mechanisms of disruption (Kruse & Gu, 2009). For example, DNA damage triggers the activation of kinases such as Chk1/Chk2 (Shieh, Ahn, Tamai, Taya, & Prives,