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Review

Small-molecule inhibitors of MDM2 as new anticancer therapeutics

Michael P. Dickens, Ross Fitzgerald, Peter M. Fischer*

School of Pharmacy & Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK

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ABSTRACT

It has long been known that traditional anticancer radio- and chemotherapies in part work through direct or indirect activation of the p53 tumour suppressor pathway. However, many of these strategies are non-selective and genotoxic. The emerging understanding of the pathways that regulate p53 has led to the notion that it should be possible to activate the p53 pathway in ways that are inherently nongenotoxic. Important targets for pharmacological interference in this respect are MDM2 and MDMX, key negative regulators of p53. Genetic and pharmacologic studies suggest that blocking the physical interaction of these proteins with p53, or inhibiting the catalytic role of MDM2 in tagging p53 for proteasomal degradation, both of which lead to an increase in the transcriptional activity of p53, may indeed be an efficient and safe way to eradicate tumour cells that retain wild-type p53. Here we review the rationale for such strategies, as well as the current state in the discovery and development of drugs that reactivate p53 by inhibiting its inhibitors MDM2 and MDMX. The first compounds that have been shown in model systems to be able selectively to kill cancer cells in this way are now entering clinical trials and the promise of MDM2 inhibitors as a new therapeutic anticancer modality should therefore become clear in the not-too-distant future.

1. Introduction

The p53 tumour suppressor protein is a transcription factor that is activated in response to cellular stress. Depending on the severity of the threat to genome integrity, p53 then imposes cell cycle arrest or apoptosis. Because p53 has strong growth-suppressive activity, it must be tightly regulated to allow normal cells to function. This is achieved to a large extent by a protein known as murine double minutes-2 (MDM2), so called because its gene was first discovered in DNA associated with paired acentric chromatin bodies, termed double minutes, in spontaneously transformed mouse 3T3 fibroblasts [1]. The corresponding human protein is sometimes referred to as HDM2 but here we shall use the abbreviation MDM2 regardless of species.

MDM2 regulates p53 in at least three different ways, *i.e.* at the levels of p53 function, protein stability, and subcellular location. MDM2 forms a protein–protein interaction with the N-terminal transcription activation domain of p53, thus blocking p53 transcriptional activity [2,3]. Furthermore, MDM2 is the E3 ubiquitin ligase that promotes ubiquitin-dependent proteasomal degradation of p53 [4,5]. Finally, MDM2 causes nuclear export of p53 into the cytoplasm of the cell, moving p53 away from its site of action [6].

While MDM2 controls the protein levels of p53, it is itself under transcriptional control of p53 and the two are thus linked in a tight autoregulatory feedback loop [7,8]. Depending on the nature of genotoxic or nongenotoxic stress a cell may experience, the negative regulation of p53 by MDM2 is interrupted in several different ways. Most importantly, the functions of MDM2 in p53 suppression are inhibited upon association with the ARF protein, an alternative transcript of the *INK4a/ARF* tumour suppressor locus, which is induced upon oncogenic stress (reviewed in [9]). Similarly, MDM2 is inhibited upon ribosomal stress by the ribosomal proteins L5, L11, and L23 [10,11]. Furthermore, MDM2 is regulated through post-translational modifications, including autoubiquitinylation [12], sumoylation, and multi-site phosphorylations by a range of kinases, especially the DNA damage-induced kinases (reviewed in [13]).

MDMX (also known as MDM4) is a nonredundant homologue of MDM2 that also regulates p53 [14] and is overexpressed in many cancers [15]. Unlike MDM2, however, MDMX expression is not regulated by p53 and MDMX is thus not part of the negative feedback loop with p53. MDMX also lacks intrinsic ubiquitin ligase activity but is itself a target for MDM2 ubiquitinylation. It forms heterodimers with MDM2, which enhances the ability of MDM2 to induce p53 degradation [16]. MDMX binds p53 at the same site and with similar affinity as MDM2 and in so doing blocks p53 transcriptional activity.

The functions of p53 are ablated in all cancers as a means of evading apoptosis, either by disabling p53 directly through mutation or deletion, or indirectly by alterations of various components

* Corresponding author. Tel.: +44 0115 846 6242; fax: +44 0115 951 3412.
E-mail address: peter.fischer@nottingham.ac.uk (P.M. Fischer).

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of the pathways that regulate p53 [17]. About half of all cancers retain wild-type p53 [18] and in these the normal regulation of p53 is sometimes disrupted through direct overexpression of MDM2 (in *ca.* 7% of cancers [19]). MDM2 overexpression due to gene amplification is especially frequent (*ca.* 30%) in human osteogenic sarcomas and soft tissue sarcomas [20].

Because of the central role of p53 in tumour suppression, nongenotoxic therapeutic strategies that activate p53 in one way or another are highly desirable. Depending on p53 status this should be able to be achieved in various ways. For example, proof-of-concept studies have shown that mutant p53 might be able to be stabilised or otherwise reactivated pharmacologically [21–23]. In tumours that retain a functional p53 pathway, on the other hand, preventing p53 degradation is an attractive option.

There are many potential therapeutic targets within the p53 pathway, downstream of the stress response, which offer the possibilities of nongenotoxic p53 activation and bypassing the particular defects that could render an upstream target ineffective. The MDM2–p53 regulatory system is one such target. Modulation of this system with small molecules is a very active area of research. Here we review current progress in the development of small molecules that inhibit the MDM2–p53 protein–protein interaction or the ubiquitin ligase activity of MDM2.

2. Target rationale and therapeutic window

The key question for any therapeutic strategy that aims to activate the p53 response is whether of not this will result in a selective effect on tumour cells as opposed to the cells of healthy tissues. Such specificity of p53 to kill tumour cells, but not normal cells, appears to underlie the safety of p53 gene therapy, which has gained approval in China and is now being developed elsewhere [24–29]. It has been shown that mice with a hypomorphic *MDM2* allele produce only about 30% of the normal level of MDM2 and exhibit increased transcriptional and functional activation of p53 [30]. The effects of p53 under these circumstances are not lethal as one might expect, although the animals are small and show p53-dependent apoptosis of lymphoid cells. Nevertheless they are viable, do not age prematurely, and are resistant to tumour formation [31].

Similarly, *in vivo* suppression of MDM2 using antisense oligonucleotides has been demonstrated to result in therapeutic antitumour effects without overt toxicity (reviewed in [32]). From these and other results [33] it is clear that the p53 pathway differs significantly in normal and p53 wild-type cancer cells and that the latter are selectively sensitive to increases in p53 effector functions. This notion is enhanced by the results of extensive pharmacological studies, especially those using the nutlin pioneer MDM2 inhibitors (discussed in more detail below), which also suggest that cancer cells are more susceptible to proapoptotic effects of p53 than noncancerous cells (reviewed in [34]).

3. The p53–MDM2 interaction

Prior to elucidation of the structure of the p53–MDM2 interaction it was thought that protein–protein interactions could not be effectively inhibited with membrane-permeable and otherwise drug-like small molecules because of the extensive size and poor definition of protein interfaces. The X-ray crystal structure of a complex between the N-terminal domain of MDM2 and a 12mer peptide encompassing residues 16–27 of the p53 transactivation domain showed that the bulk of the p53–MDM2 interaction in fact involved just three lipophilic residues of p53, buried in a well-defined hydrophobic surface cleft in MDM2, of a size that could clearly be fully occupied by a small molecule [2]. This observation has helped to reshape our perception of protein–protein interactions as drug targets, since in many cases these contain so-called hot spots, where the binding energy of protein–protein interactions is concentrated [35].

3.1. Early work with peptide antagonists of the p53–MDM2 interaction

A detailed discussion of peptide and peptidomimetic approaches to modulate the p53–MDM2 interaction has been provided elsewhere [36–38] and we shall only summarise in outline some of the early peptide optimisation studies that defined the pharmacophore model which provided the platform for subsequent development of nonpeptide inhibitors.

Initially, screening of phage-displayed peptide libraries led to the discovery of a 12mer peptide MPR**F**MDY**W**EG**L**N with 28-fold potency increase compared to the corresponding p53 sequence ^{16}QET**F**SDL**W**KL**L**F^{27} [39]. Interestingly, only the three key interacting residues (bold type in preceding sequences) were conserved between these peptides and the basic pharmacophore feature of all potent ligands is indeed three suitably oriented hydrophobic groups. Next, artificial amino acids were used to explore conformational features. This led to the development of a highly optimised 8mer peptide that inhibited the p53–MDM2 PPI with low nanomolar potency, which represented a >1700-fold increase in affinity compared with the 12mer p53 peptide [40].

Incorporation of a chloro group at the indole C6 position of the key Trp residue showed that better occupancy of the binding site compared to the cognate ligand could be achieved with substantial potency gains. The effect of introducing helix-stabilising residues showed the importance of a rigid scaffold in presenting the key residues in a way that results in optimal shape complementarity with the binding site. Again this feature was subsequently recapitulated with nonpeptidic inhibitors. The increases in affinity brought about by inclusion of charged nonnative residues indicated that further polar contacts not present in the native system could be made outside of the main binding cleft.

A complex crystal structure of this high-affinity 8mer peptide bound to MDM2 was solved recently [41] and shows that the peptide does indeed bind in the expected manner (Fig. 1). The structural features of this peptide have been inherited by subsequent small-molecule inhibitors, the best of which are those that mimic the peptide most closely. The optimised peptide also provided pharmacological target validation, since it was somewhat permeable and thus able to reach its target MDM2 in intact cells. It was observed to induce apoptosis selectively in MDM2-overexpressing cancer cells *via* nongenotoxic p53 activation [42].

3.2. Small-molecule p53–MDM2 antagonists

Because of their central role as pioneers for protein–protein interaction drug target modulators in general, inhibitors of the p53–MDM2 interaction have been reviewed extensively. We do not intend to duplicate these efforts here but direct the interested reader to some of the most recent reviews [43–47]. One of these gives an up-to-date summary, covers some 20 distinct classes of small-molecule p53–MDM2 inhibitors, and assesses critically to what extent these have been validated, *i.e.* which can be regarded as genuine p53–MDM2 inhibitors and which operate to block MDM2 functions by different mechanisms [45].

Of the small-molecule inhibitor series described to date, three are of particular importance. The nutlins [48], the benzodiazepinediones [49–53], and the spiro-oxindoles [54,55] (important representative members from these series are shown in Fig. 2) all bind MDM2 with low nanomolar affinity and induce cancer cell apoptosis in a p53-dependent manner. Typically these compounds