REVIEWS

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Will Therapies that Target Tumour Suppressor Genes be Useful in Cancer Treatment?

Czy terapia celowana prowadząca do utraty funkcji genów supresorowych będzie miała zastosowanie w leczeniu nowotworów?

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Human cancers represent one of the biggest challenges for modern societies. By 2020, cancer deaths worldwide could reach 10 million. Therefore, one of the important aims of science research is the improvement of anti-malignant treatment options to mitigate cancer-related morbidity and mortality. This essay summarises current trends and future directions of target tumour suppressor genes therapies based on an example of one of the most promising targets, p53 **(Adv Clin Exp Med 2013, 22, 6, 861–864).**

Key words: children, cancer, leukaemia, genes, targeted therapy.

Streszczenie

Choroby nowotworowe stanowią coraz większy społeczny problem. Uważa się, że do 2020 r. mogą być odpowiedzialne za śmierć nawet 10 milionów osób na świecie. Dlatego tak ważne jest poprawienie skuteczności leczenia przeciwnowotworowego, co w następstwie może znacznie zmniejszyć śmiertelność. Praca przedstawia aktualne trendy oraz przyszłe kierunki terapii celowanej genów supresorowych na przykładzie białka p53, rozpatrywanego jako najbardziej obiecujący cel terapii antynowotworowej **(Adv Clin Exp Med 2013, 22, 6, 861–864).**

Słowa kluczowe: dzieci, nowotwór, białaczka, geny, terapia celowana.

Human cancers represent one of the biggest challenges for modern societies. In 2010, there were more than 324.500 new cancer cases diagnosed in the United Kingdom [1]. By 2020, cancer deaths worldwide could reach 10 m. Therefore, one of the important aims of science research is the improvement of anti-malignant treatment options to mitigate cancer-related morbidity and mortality.

Human cancer develops via a multistep process reflective of genetic alterations that influence key cellular pathways involved in growth and development. This process, involving tumour suppressor gene inactivation and oncogene activation, leads to uncontrolled clonal proliferation [2]. Effective treatments targeting oncogenes or their protein products have so far proven to be elusive in spite of combined efforts of many researches [3]. The appearance of genetic mechanisms of drug resistance has become the “Achilles heel” of cancer therapy. There is a great need for combinations of treatments acting through different mechanisms to prevent the development of resistant disease. Therefore, tumour suppression genes therapies offer promise in view of their high specificity and the possibility of using them in combination with other therapies.

This essay summarises current trends and future directions of target tumour suppressor genes therapies based on an example of one of the most promising targets, p53 (tumour protein 53).

Tumour Suppressor Genes

Tumour suppressor genes are genes that decelerate cell division, repair DNA aberrations, or instruct cells when to induce cell death (apoptosis or programmed cell death). Homozygous inactivation of tumour suppressor genes can arise through point mutation, deletion, chromosomal aberration or dysregulated expression [3]. A mutation in 1 allele is another mechanism responsible for inactivation of tumour suppressor genes. A mutation of one allele alone (heterozygous mutation) may also lead to inactivation of tumour suppressor gene, when the protein encoded by the mutant allele exerts a dominant-negative effect, i.e. forms heterodimers with the wild-type protein, thereby causing its functional inactivation [3]. It has been proven that restoration of a tumour suppressor genes’ function can resume the normal phenotype and/or cause the death of the malignant cell [4].

p53

The p53 transcription factor was 1st described as an oncogene in 1979 [2]. Then, it has been rediscovered as a tumour suppressor gene in 1989 and became one of the most profoundly studied proteins to date [5].

It has been well known that the p53 protein is relatively unstable and has a short half-life ranging from 5 to 30 min, which makes it undetectable by immunohistochemistry. In contrast, mutant p53 has a much longer half-life, and, therefore, accumulates in the nucleus creating a stable target for immunohistochemical detection [6].

Cellular stress (including genotoxic damages), oncogene activation, and hypoxia stabilise p53 and lead to an increase in its transcriptional activity through posttranslational modifications by phosphorylation, acetylation, ubiquitination, and methylation. The inhibition of p53 interaction with MDM2 (mouse double minute 2 homolog), an ubiquitin E3 ligase, is an essential step in the activation of p53. MDM2 along with its partner MDM4 (mouse double minute 4 homolog) ubiquitinates p53 and promotes the proteasome-mediated degradation of p53 [7]. MDM2 has also been proven to be a downstream target of p53. This ensures that p53-induced responses are regulated [7]. Once activated, p53 plays a pivotal role in tumour suppression by inducing growth arrest, activating apoptosis, senescence, and blocking angiogenesis. While p53 has been mainly recognised as a transcription activator, it can also inhibit the activity of genes [8]. The frequent loss of p53 activity in human tumours has turned researchers’ attention to p53 as an appealing therapeutic target for anticancer drug discovery [6].

The Possible Strategies for the Distinct Types of p53 Dysregulation in Human Cancers

The reinstatement of natural tumour suppressive pathways in cancer cells remains an attractive treatment strategy. Using gene suppression techniques enables the reinstatement of previously inactivated tumour suppressor pathways.

p53 Mutations

Mutations of the *TP53* gene are the most common molecular alterations in human cancer, occurring in ~50% of human cancers [5]. Cancers expressing mutant p53 may or may not retain a wild-type (wt) allele. Some mutations may suppress the normal function of wt p53. Therefore, the functional reactivation of the mutant protein became a focal point in the development of targeted therapies for these tumours.

In 2009 Lambert et al. showed that restoration of wild-type p53 expression triggers cell death and eliminates tumours *in vivo*. PRIMA-1 is a small molecule causing the functional reactivation of mutant p53’s transcriptional function through the covalent binding of its core domain [9]. The identification of mutant p53-reactivating small molecules such as PRIMA-1 opens possibilities for the development of more efficient anticancer drugs. These findings might facilitate the design of more potent and specific mutant p53-targeting anticancer drugs.

p53 Loss

Both partial and full homozygous deletion of the *TP53* gene can lead to p53 loss, which has been observed in many different tumour types [10]. Besides, the combination of a p53 inactivating mutation and a deletion of the wild type allele (loss of heterozygosity – LOH) can lead to loss of p53 function. As p53 function is lost in many tumour types, the strategy of choice in these circumstances is gene therapy enabling the restoration of p53 function by reintroducing wt p53. In 1993 Fujiwara et al. successfully used retrovirus-mediated gene transfer of wt p53 into human lung cancer to suppress tumour growth *in vitro* and *in vivo* [11]. The Ad-p53 is a replication defective, p53 producing adenovirus that has been in clinical use in China since 2003. Its brand name is Gendicine or Advexin. This vector is well tolerated in patients and effective in the therapy of cancers such as head and neck cancer. There have also been reported other promising viral vectors [6]. The functional reactivation of p53 can also be achieved with reactivating molecules, as described above.

Inhibition of wt p53

Another strategy for activation of wt p53 is an inhibition of its negative regulators. The best-known natural p53 inhibitor is its downstream target MDM2. So far several inhibitors of the MDM2-p53 interaction have been described. Nutulin-3 is a small molecule that suppresses the MDM2/p53 interaction, leading to the p53 stabilisation, activation of cell cycle arrest and apoptosis (Fig. 1). It has shown encouraging cytotoxic and cytostatic effects in cancer cell lines that contain wt p53, with low toxicity [12]. In 2003 Lowe and Sherr described additional therapeutic targeting opportunities and p53 inhibition by viral oncoproteins and hypermethylation or inhibition of $p19^{Arf}$ (19 kilodalton, 169 amino acid mice protein) [13].

Chemoradiation

The activation of endogenous wt p53 can occur due to chemoradiation. Both genotoxic anticancer drugs and ionizing radiation cause the considerable DNA damage that triggers p53 activation and stabilisation. There is early preclinical data available indicating that cells or tumours with a wt p53 are more sensitive to chemoradiation not only *in vitro* cell *but in vivo* tumour models as well [6].

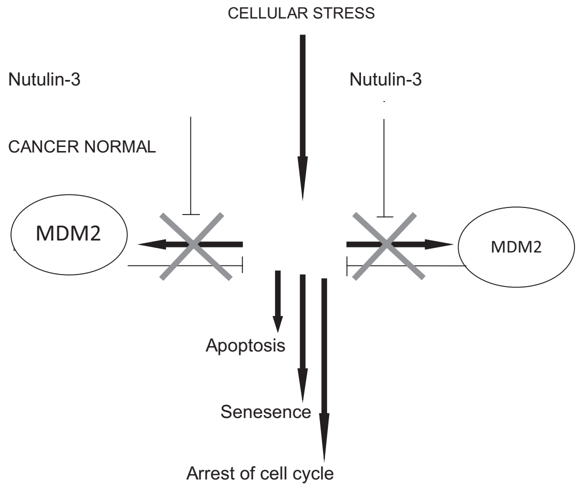

Fig. 1. The role of Nutlin-3. Nutulin-3 inhibits the interaction between MDM2 and p53, leading to the stabilisation and activation of p53 and therefore induction of senescence (biological aging), activation of cell cycle arrest and apoptosis. MDM2; mouse double minute 2 homolog, p53; tumour protein 53

Ryc. 1. Rola nutliny-3. Nutlina-3 hamuje interakcję między MDM2 i p53, prowadząc do stabilizacji i aktywacji p53, indukując tym samym biologiczne starzenie się (*senescence*) komórek, zahamowanie cyklu komórkowego i apoptozę

There are no clinical data available on the therapeutic impact of p53 restoration in human malignancy. Further optimisations are required to establish the true efficacy of various scientific strategies of tumour treatment. Researchers reported promising results regarding p53 restoration that led to the clearance of few tumour types, such as Eμ-Myc driven lymphoma and $Hras^{v12}$-driven liver carcinoma [5]. However, current *in vivo* models unable us to assess the efficacy of p53-based malignancy therapies and the adverse effects of systemic p53 restoration. In 2006 Ringshausen et al. showed that p53 is spontaneously active in MDM2-null mice and activate fatal sequelaes such as ablation of classically radiosensitive tissues [14].

Besides toxicity, it may be difficult to achieve high efficacy of p53-targeted cancer therapy. Cancer therapies may improve over the next few years but at the same time *in vivo* data suggest that the mechanisms of resistance to these targeted therapies may evolve. One of the limitations may be innate resistance to p53 restoration in view of the fact that not all malignant cells have adequate stress signals to activate a restored p53 protein. Therefore, these cells may be left undisturbed. At the same time, despite the restored p53 protein activation in a significant number of malignant cells, a cytotoxic effect may not occur [5].

Disregarding its limitations, the restoration of a functional p53 protein in human malignancies may be beneficial to patients. A better understanding of the roles of *TP53* and other tumour suppressor genes may allow scientists to design combined therapies acting through different mechanisms to prevent the development of resistant disease.

An efficient individualised cancer therapy needs a profound understanding of genetic and epigenetic alterations of each individual cancer. These therapies require rational design of combinational therapies targeting the altered molecules and signalling pathways.

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