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Method for Influencing the p53 Linkage to Target Genes

The present invention relates to a method for influencing the p53 linkage to target genes. p53 is a protein present in animal and human cells and is referred to as a "guardian of the genome". p53 is a sequence-specific transactivator which is activated in the case of DNA damage. In this form, p53 binds to promoters of target genes and activates the transcription thereof. This causes growth stand-still of the cells and subsequent repair of the DNA damage and cell death, respectively.

It has turned out that p53 has lost its transactivator activity in many tumors. This is often due to the fact that the linkage of p53 to the promoters of target genes is disturbed or it is not the desired target genes that are regulated.

Therefore, it is the object of the present invention to provide a product by which it is possible to influence the linkage of p53 to target genes.

According to the invention this is achieved by the subject matters defined in the claims.

Thus, the subject matter of the present invention relates to a method of influencing the linkage of p53 to a target gene in which the conformations of p53 and the target gene are coordinated, particularly by means of conformation modulators, and the linkage of p53 is detected directly or indirectly.

The present invention is based on the applicant's insight that p53 binding sequences in the promoters of target genes can be present not only in the "ordinary" B-duplex DNA conformation but also in non-B DNA conformations (e.g. cruciate). The applicant

also discovered that p53 identifies its binding sequences in both the B-duplex DNA conformation and in the non-B DNA conformations. However, the identification differs depending on the conformation of p53.

According to the invention these insights are used to influence to linkage of p53 to target genes by coordinating the conformations of p53 and the target genes, particularly by means of conformation modulators, and detecting the p53 linkage directly or indirectly.

The expression "p53" comprises a p53 of any kind and origin. It can have a wild-type sequence or be a mutated p53. The latter is preferably a p53 with mutated ability of DNA binding. p53 can also be present in the form of a fragment of p53 which is responsible for the DNA binding. In addition, p53 can be present in the form of a fusion polypeptide. Like any other p53 it can also be present in the form of a vector coding for it.

The term "target genes" comprises genes of any kind and origin the expression of which is regulated by p53. Examples of such genes are RGC, MCK, mdm2, cyclin G, synthetic p53 reporter genes, p21 and bax, p21 and particularly bax being preferred. p21 is held responsible for the growth stand-still of the cell caused by p53 and bax is held responsible for the cell death caused by p53. In particular, the expression "target genes" comprises the promoter sequences thereof and more particularly p53 binding sequences thereof. The target genes may be present in any DNA conformation. They can be present in cells, particularly tumor cells, or occur in isolated fashion. The target genes can also be present in connection with further sequences, particularly with those coding for a reporter protein.

The expression "conformation modulators" comprises substances of any kind which can cause a conformational change of a nucleic acid. In particular, substances are concerned which can convert

a DNA from the B-duplex DNA conformation into a non-B DNA conformation or vice versa. Examples of such substances are intercalating substances. The expression "conformation modulators" also comprises substances of any kind which can effect a conformational change of p53. Examples of such substances are those which can modify the carboxy-terminal regulator region of p53, e.g. antibody pAB 421.

The expression "direct or indirect detection of p53 linkage" comprises any detection for such a linkage. Examples of a direct detection comprise methods by means of which the p53 linkage to DNA can be shown. Indirect detections include methods by means of which it is possible to show the consequences of a p53 DNA linkage, e.g. the regulation of the expression of reporter genes and/or biological consequences, such as the growth stand-still of cells or the death thereof.

A method according to the invention can be carried out as usual. For example, p53 binding sequences in the B-duplex DNA conformation and in a non-B DNA conformation, respectively, can be incubated with p53 and optionally conformation modulators and the DNA binding of p53 is detected directly. It is possible to incubate cells, particularly tumor cells, having disturbed DNA binding of p53 with conformation modulators and upon irradiation of the cells the DNA binding of p53 is detected indirectly via the growth stand-still and the death of the cells, respectively.

By means of the present invention it is possible to influence the p53 linkage to target genes. This can be used to correct a disturbed DNA linkage of p53, which often exists in tumor cells. Furthermore, the DNA binding specificity of p53 can be influenced so as to regulate or control certain desired target genes. Thus, the present invention can be used at least as an accompanying treatment measure to combat tumoral diseases.

The present invention also offers the possibility of discovering substances which can be suitable as conformation modulators of

p53 and/or its target genes. For this purpose, the method according to the invention is carried out to the effect that in place of known conformation modulators unknown ones are used and the known conformation modulators are optionally used as controls.

Brief description of the drawings:

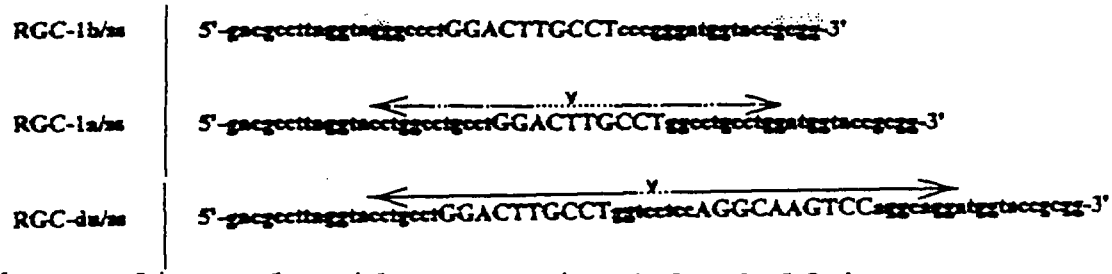
Figure 1 shows the DNA conformations of oligonucleotides which contain p53 binding sequences of RGC,
figure 2 shows the DNA binding of p53 to oligonucleotides by p53 binding sequences of RGC, the oligonucleotides being present in various DNA conformations. Lanes 1 and 2 represent controls,
figure 3 shows the DNA conformations of oligonucleotides which contain p53 binding sequences of the sequence found by Hupp,
figure 4 shows the DNA binding of p53 to oligonucleotides by p53 binding sequences of the Hupp sequence, the oligonucleotides being present in various DNA conformations.

The invention is explained by the below example.

Example: p53 linkage to a target gene

(a) p53 linkage to p53 binding sequences of RGC

p53 was isolated as usual from Sf9 insect cells which were infected with recombinant wild type p53-expressing baculoviruses. p53 had a purity of over 80 % in an SDS polyacrylamide gel. p53 was used in a linkage reaction with oligonucleotides. For preparing these oligonucleotides, the below oligonucleotides were used as a basis:



These oligonucleotides contain 1-2 "halfsites" of the p53 binding sequences of RGC and in each case identical terminal sequences at their 5' ends and 3' ends. The oligonucleotides were used in an "annealing" reaction with the below oligonucleotide which has complementary sequences with respect to the terminal sequences:

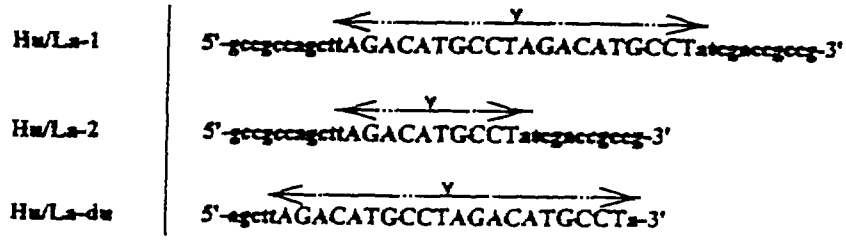


Oligonucleotides were obtained which are present in the duplex-B DNA conformation (structure II) and in the non-B DNA conformations, respectively (structures I and III, respectively) (cf. figure 1). These oligonucleotides were terminally labeled radioactively and used in the binding reaction with p53. The reaction lasted 30 minutes at room temperature. The reaction products were subjected to polyacrylamide gel electrophoresis.

It turned out that p53 binds to the oligonucleotides of the three DNA conformations, although to differing degrees. The strongest bond is found in the case of a non-B DNA conformation (structure I), while the bond to the duplex-B DNA conformation (structure II) and to another non-B DNA conformation (structure III), respectively, is weaker (cf. figure 2). Thus, it becomes evident that the p53 linkage to a target gene can be influenced by a conformational change of the target gene.

(b) p53 linkage to p53 binding sequences of the Hupp sequence

In accordance with the description of (a), p53 was used in a binding reaction with oligonucleotides. For the preparation thereof, the below oligonucleotides were used as a basis:



They contain 1-2 "halfsites" of the p53 binding sequences discovered by Hupp. These p53 binding sequences distinguish themselves in that p53 can bind thereto only after its activation by the antibody PAb 421. The oligonucleotides were used in an "annealing" reaction with the oligonucleotide "lock" indicated in (a). Oligonucleotides were obtained which are present in the duplex-B DNA conformation (Hu/La-du/ds) and in the non-B DNA conformations, respectively (Hu/La-2 and Hu/La-1, respectively) (cf. figure 3).

It turned out that p53 binds to the oligonucleotides of the three DNA conformations, although to differing degrees. p53 binds to the oligonucleotide in the duplex-B DNA conformation only after the activation of p53 by the PAb 421 antibody, while its linkage to non-B DNA conformations takes place without modification of p53 (cf. figure 4). On the other hand, the linkage of PAb 421 to p53 effects an inhibition of the p53 linkage to this oligonucleotide in the non-B DNA conformation. Hence it becomes evident that the p53 linkage to a target gene can be influenced by conformational changes of the target gene and of p53.

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Claims

1. A method for influencing the p53 linkage to a target gene, wherein the conformations of p53 and the target gene are coordinated and the p53 linkage is detected directly or indirectly.

2. The method according to claim 1, wherein the coordination of the conformations is effected via one or several conformation modulators.

3. The method according to claim 1 or 2, wherein p53 is a mutated p53.

4. The method according to claim 3, wherein the mutation of p53 relates to the ability of DNA linkage.

5. The method according to any one of claims 1 to 3, wherein the target gene is p21 or bax.

6. The method according to any one of claims 2 to 5, wherein the conformation modulator relates to p53.

7. The method according to claim 6, wherein the conformation modulator relates to the carboxy-terminal regulator region of p53.

8. The method according to claim 6 or 7, wherein the conformation modulator is the antibody PAb 421.

9. The method according to any one of claims 2 to 5, wherein the conformation modulator relates to the target gene.

10. The method according to claim 9, wherein the conformation modulator is an intercalating substance.

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11. The method according to any one of claims 2 to 5, wherein the conformation modulator is a substance to be determined.

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