DEVELOPMENT OF GENETIC ENGINEERING TECHNIQUE USING SUPERHELIX DNA BINDING PEPTIDE

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Summary of the Topic

The present technique of using a peptide (SRD) that recognizes and binds a specific superhelix DNA structure on an activated gene is a genetic engineering technique based on novel principles, and similar techniques have not yet been developed.

Although a long sequence composed of 137 amino acid residues was focused upon in developing the superhelix DNA binding peptide in the technique of patent application, it was found in later research that a shorter synthetic peptide sequence (KEK) of 27 residues had sufficiently high specificity. The present research establishes the foundation of a technique for genetic engineering of a superhelix DNA by searching for an active peptide with higher specificity with the KEK as the lead sequence, developing an efficient introduction method into cells, and determining localization within cells.

Technical Field of the Topic

Medical technology, drug discovery

1. Advantages of the Developed Technique

The present technique of using the transcriptionally active domain (SRD) of a protein that recognizes and binds a characteristic superhelix DNA structure on an activated gene is a genetic engineering technique based on novel principles, and since similar techniques thereto have not yet been developed, the technique is extremely advantageous.

Further, there is a strong possibility that in the future, a series of related techniques will be developed based on the present technique.

A patent application for active peptides that include an SRD and an SRD derivative has already been submitted. Creation of a negative superhelix DNA binder (drug) or induction of specific chemical reactions in a transcriptionally active region within a cell nucleus using the above is also included in the patent.

2. Marketability and Future Prospects for the Technique

The HIV-1 virus DNA is incorporated in a transcriptionally active chromatin region of infected

cells. As a result, the virus RNA is efficiently transcribed and a significant proliferation of the infectious virus occurs.

The protein that targets the virus DNA to the transcriptionally active region within cell nuclei is LEDGF/p75. It has been proven that LEDGF/p75 and SBP75 are the same protein and that a new domain SRD that selectively binds to a superhelix DNA is indispensable for the targeting to the transcriptionally active region (Tsutsui, KM, et al. Nucleic Acids Research, doi: 10.1093/nar/gkr088, 2011). Further, it was revealed in the same paper that a core region derivative (KEK peptide) of the SRD competitively inhibits the binding of SPB75/LEDGF/p75 with superhelix DNA.

In the field of HIV-1 viral infections, the KEK peptide enables the development of treatment drugs with new points of actions that are completely different from drugs developed in the past.

Further, by using the KEK peptide, it becomes possible to insert a target gene in a transcriptionally active genome region, and an effective gene therapy technique becomes possible.

3. Objectives and Needs

Although a superhelix DNA recognition domain (SRD) composed of the 137 amino acid residues that are in a superhelix DNA binding protein SPB75 was the focus in developing the technique of the patent application, it was found in later research that a synthetic peptide sequence (KEK) of a shorter 27 residues had sufficiently high specificity.

In seeking to achieve introduction into cells, shorter active sequences are more advantageous. The present experimental research is establishing the foundation of a technique for genetic engineering of superhelix DNA by searching for an active peptide with higher specificity with the KEK as the lead sequence, developing an efficient introduction method into cells, and determining localization within cells.

4. Future Plans

• Synthesize many peptides in which the sequence of KEK has been changed to investigate binding with a superhelix DNA, and find a peptide sequence for which there is a high specificity and that bonds more strongly.

• Synthesize active peptides that are fluorescently labeled, find an efficient cell introduction method, and observe the localization within cells. This research has the possibility of leading to an HIV infection inhibitor utilizing a novel principle, which competitively inhibits the incorporation of the HIV virus into the cell DNA.

• Develop a technique of selectively incorporating foreign genes into transcriptionally active genome regions by cotransfecting an HIV integrase expression plasmid in which the active peptides are fused and a lentivirus vector into which the genes to be introduced are incorporated.

5. Patent

Title of Patent	Negative Superhelix DNA Binding Domain		
Application Number	Japanese Patent Application No. 2008-188287	Application Date	July 22, 2008
Applicant	National University Corporation Okayama University		
Inventors	Ken Tsutsui, Kimiko Tsutsui, Kuniaki Sano, Tadashi Miyamoto		
Summary	Creation of a negative superhelix DNA binder (drug) or evocation of specific chemical reactions in a transcriptionally active region within a cell nucleus using the domain SRD of the protein SBP75 that binds with a specific negative superhelix DNA or an active peptide that includes a derivative of the SRD.		

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PRESS RELEASE

Okayama University

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Does the AIDS Virus Seek Out Twisted DNA?

A research group led by Professors Kimiko Tsutsui and Ken Tsutsui of Okayama University's Graduate School of Medicine, Dentistry and Pharmaceutical Sciences has discovered a protein that recognizes and binds to the twist (superhelix) of a DNA and has ascertained a short amino acid sequence (SRD) that serves such a function. The research will be published in the British specialized journal Nucleic Acids Research.

It has been discovered that such a protein is the same as the protein that is necessary for the human immunodeficiency virus (HIV) to enter the DNA of cells, and the possibility that superhelix DNA is related to the infection mechanism of HIV has surfaced. It is hoped that a curative drug with a new action mechanism will be developed in the future using SRD.

• The research group led by Professors Tsutsui discovered that the protein in the human cell which the AIDS virus (HIV) uses in entering the cell's DNA binds preferably with a twisted DNA (superhelix DNA), and have proposed a new infection mechanism of HIV.

• The professors discovered over twenty years ago that there was a protein within cell nuclei

which selectively binds with superhelix DNA, and have now proven that such a protein (SBP75) is the same as a cellular factor (LEDGF) that is necessary for HIV to infect a cell.

• Over 20,000 genes are stored in the DNA of a cell nucleus. However, not all genes are always transcriptionally active. The genes that are transcriptionally active are gathered at so-called transcription factories, and the DNA at such locations becomes twisted.

• From the observation that SBP75 gathers around an activated gene, it is believed that SBP75 performs a role of finding a twisted DNA that is bound to other proteins and sending a protein that is necessary for the gene to be transcribed to a transcription factory.

• It is known that HIV selectively enters genes that are transcriptionally active, and such a phenomenon is easily explained by the properties of SBP75.

• Another significant achievement has been the discovery of the previously unknown component (SRD) of SBP75 that directly attaches to the superhelix DNA, discovered by dissecting SBP75 molecules in detail.

• It has also been discovered that a short sequence (KEK) that mimics the amino acid sequence of SRD is able to identify a superhelix DNA. It is possible to create curative drugs with a new action mechanism using KEK.

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