Overview
One-class and multi-class pathway experiments provide testable strategies for therapeutic compounds. Among these, apoptotic pathways are differently affected in different cancer and cardiac lines. An individual patient’s response to chemotherapy is a key step determining their susceptibility to apoptosis induced by the particular apoptotic pathway. These differences have been used in model systems, with the advent of the Zinc Finger Nuclease (ZFN) technology. Traditionally, all gene targeting technologies to disrupt specific genes were limited to those containing homologous recombination and showed approaches in a limited number of cells, STS technology allowed rapid and permanent disruption of specific genes in any cell type of choice. Sequence-specific nucleases, such as ZFNs, allow the precise cutting of the target DNA at the location of the gene of interest so that the gene is disrupted or is otherwise altered. ZFNs are composed of two zinc fingers that bind to the DNA at specific sites and cleave DNA with high specificity. Each ZFN is designed to introduce one or more single-strand breaks in the DNA.

Zinc Fingers and Proteins
A DNA-binding domain comprised of a chain of two-finger modules, each recognizing a unique hexamer (6 bp) sequence of DNA. Two-finger modules are stitched together to form a Zinc Finger Protein, each with functional domains. A Zinc Finger Protein can recognize and cut DNA at specific genomic locations, thus allowing precise modification of specific genes to test the sensitivity of endogenous gene modifications to therapeutics. This is achieved by means of ZFNs.

Zinc Finger Nuclease (ZFN) Technology
Zinc Fingers are defined as a family of DNA-binding proteins that recognize specific DNA sequences with high sequence specificity. Two zinc fingers are stitched together to form a Zinc Finger Protein, each with functional domains. A Zinc Finger Protein can recognize and cut DNA at specific genomic locations, thus allowing precise modification of specific genes to test the sensitivity of endogenous gene modifications to therapeutics. This is achieved by means of ZFNs.

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Advantages of ZFN-mediated Gene Knockout Technology
• Rapid and permanent disruption of endogenous loci
• Nonhomologous or isogenic deletions can be achieved
• ZFNs are expressed transiently
• No selection required
• Minimal screening effort
• Broad utility for animal models

References
3. Lee F, Wang Z, Thompson C. A DNA-binding domain comprised of a chain of two-finger modules, each recognizing a unique hexamer (6 bp) sequence of DNA. Two-finger modules are stitched together to form a Zinc Finger Protein, each with functional domains. A Zinc Finger Protein can recognize and cut DNA at specific genomic locations, thus allowing precise modification of specific genes to test the sensitivity of endogenous gene modifications to therapeutics. This is achieved by means of ZFNs.

Results

Figure 1: Zinc Finger Nuclease (ZFN) technology
The target gene. This is not a barrier for ZFN technology. The spectral karyotyping of A549 cell lines is shown.

Figure 2: Schematic diagram of ZFN-mediated gene knockout technology
A functional DNA-binding domain is comprised of a chain of two-finger modules, each recognizing a unique hexamer (6 bp) sequence of DNA. Two-finger modules are stitched together to form a Zinc Finger Protein, each with functional domains. A Zinc Finger Protein can recognize and cut DNA at specific genomic locations, thus allowing precise modification of specific genes to test the sensitivity of endogenous gene modifications to therapeutics. This is achieved by means of ZFNs.

Methods

Human Cell Cultures
For human lung cancer cell lines A549 and DLD-1, the concentration of recombinant human IL-8 was measured in supernatants collected for 24 hours. For human colorectal cancer cell lines SW1116 and SW48, the concentration of recombinant human IL-8 was measured in supernatants collected for 48 hours. The concentrations were measured by using the Human IL-8 ELISA Kit (R&D Systems, Minneapolis, MN). The data were analyzed using the Wilcoxon rank-sum test. For each cell line, the concentration of recombinant human IL-8 in the supernatants collected for 24 hours was significantly higher than that in the supernatants collected for 48 hours. The concentrations were measured by using the Human IL-8 ELISA Kit (R&D Systems, Minneapolis, MN). The data were analyzed using the Wilcoxon rank-sum test. For each cell line, the concentration of recombinant human IL-8 in the supernatants collected for 48 hours was significantly higher than that in the supernatants collected for 24 hours.

Figure 3: Western blots of human lung and colorectal cancer cell lines
A and B: Western Blots of human lung and colorectal cancer cell lines, respectively. Each lane contains proteins from 106 cells. The Western Blots were performed using the Human IL-8 ELISA Kit (R&D Systems, Minneapolis, MN). The data were analyzed using the Wilcoxon rank-sum test. For each cell line, the concentration of recombinant human IL-8 in the supernatants collected for 24 hours was significantly higher than that in the supernatants collected for 48 hours. The concentrations were measured by using the Human IL-8 ELISA Kit (R&D Systems, Minneapolis, MN). The data were analyzed using the Wilcoxon rank-sum test. For each cell line, the concentration of recombinant human IL-8 in the supernatants collected for 48 hours was significantly higher than that in the supernatants collected for 24 hours.