METHODS IN MOLECULAR BIOLOGY

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Suicide Gene Therapy

Methods and Protocols

Edited by

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Preface

The concept of suicide gene therapy most likely originated in 1986 with the work of Frederick Moolten at the Hubert H. Humphrey Cancer Research Center at Boston University. He exposed neoplastic BALB/c murine cell lines carrying the herpes simplex virus thymidine kinase gene to the herpes thymidine kinase-specific substrate 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy] methyl}guanine, which inhibited the clonogenic potential of the cells in vitro. Tumors produced by these cell lines in BALB/c mice underwent complete regression following exposure to this drug.

Subsequently, in 1988, Ronald Evans and collaborators at the Salk Institute in La Jolla used a procedure to kill transfected cells in culture or to selectively delete immune cells in transgenic mice upon administration of acyclovir or 1-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl)-5-iodouracil (FIAU). In 1991, Knudsen and Karlström at the University of Copenhagen used the *Escherichia coli relF* gene driven by *lac*-derived promoters to devise a biological containment system for genetically engineered bacteria. In the same year, the Pardoll laboratory at Johns Hopkins University in Baltimore utilized the HSV-*tk* + ganciclovir system in tumor cell vaccines as a means to eliminate the cells in case the immune system was unable to clear them. Klatzman and colleagues at the Hôpital de la Pitié-Salpêtrière, Paris, in 1992, employed the HSV-*tk* + ganciclovir system to kill liver tumor cells in vivo. They also engineered CD4+ T-lymphocytes to express HSV-*tk* under the control of the HIV promoter (5′-LTR) and showed the elimination of these cells upon HIV infection. The Blaese laboratory at the National Cancer Institute showed in 1994 that tumor cells expressing the cytosine deaminase gene are eliminated in vivo by the administration of 5-fluorocytosine, which is converted to the toxic compound, 5-fluorouracil.

This volume includes the methods used for most of the recent approaches to suicide gene therapy of cancer. It also contains chapters describing methods to improve the safety of cell therapy and those utilizing bone marrow mesenchymal cells. The goal of cancer treatment is to eliminate, or at least to greatly reduce, the number of cancer cells without harming normal cells. In addition to utilizing cell surface markers overexpressed on cancer cells, targeted suicide gene therapy exploits promoters that are specific to cancer cells, thereby ensuring (or greatly increasing the likelihood) that the therapeutic gene is expressed only in cancer cells.

In the first chapter, we outline the origins of the different systems used for suicide gene therapy. In Chapter 2, Fehse, Miletic, and collaborators provide guidelines for the preparation of high-titer 3rd-generation lentiviral vectors that encode a genetically improved HSV-*tk* version (TK.007) and its application in vitro and in vivo. The success of suicide gene therapy can be greatly enhanced by the use of tissue-specific or tumor-specific gene expression and efficient gene delivery. Thus, Chung and Hsieh and co-authors provide the details of osteonectin promoter-mediated suicide gene therapy of prostate cancer in Chapter 3. Pedroso de Lima and collaborators describe in Chapter 4 the methods for the use of the HSV-*tk*/ganciclovir system to achieve antitumor activity both in cultured oral cancer cells and in orthotopic and subcutaneous murine models of oral squamous cell carcinoma, using ligand-associated lipoplexes for enhancing therapeutic delivery.

Although chimeric antigen receptor (CAR)-redirected T-cells can be used for the treatment of cancers, they can have adverse effects such as the cytokine release syndrome and off-target effects that can cause organ damage. In Chapter 5, Di Stasi and co-authors describe an inducible caspase 9 suicide gene system that can eliminate a large percentage of CAR T-cells when necessary. Altaner and Altanerova outline their methods to prepare exosomes derived from human mesenchymal stem cells engineered to express cytosine deaminase and uracil phosphoribosyl transferase mRNA, and their use in inhibiting the growth of cancer cells following the administration of 5-fluorocytosine as a prodrug (Chapter 6). In Chapter 7, Saydam and colleagues describe a similar system, which also expresses the green fluorescent protein in HEK293T cells, to produce extracellular vesicles that they have used to inhibit the growth of glioblastoma cell lines and spheroids, and glioblastoma tumors in vivo.

Viral vectors with suicide genes are very difficult to produce, because trace amounts of toxins (such as diphtheria toxin or the enzyme barnase) can kill the cells used for viral vector production. In Chapter 8, Chen describes a method to overcome this problem. In this method, insect cells are used, since mammalian introns are not recognized by these cells, and the open reading frames of the toxic genes are not broken up. Thus, the insect cells do not produce the toxins but generate normal levels of baculovirus and adeno-associated viral vectors carrying the toxin genes. Bhatia and Shi (Chapter 9) describe a vector containing resveratrol-responsive CC(A+T rich)6GG (referred to as a "CArG box") elements from the promoter of the early growth response protein 1 (Egr-1) and the GADD45α open reading frame. After delivery into lung cancer cells and treatment with resveratrol, the vector expresses GADD45α, leading to cell cycle arrest.

Suicide gene expression may be used as a safety mechanism in regenerative medicine. Unwanted multipotent stem cells could thus be removed before they cause side effects, such as teratoma formation. The location of such cells can be monitored by imaging approaches. Himmelreich and co-authors describe in Chapter 10 how therapeutic cells can be engineered to express a suicide gene as well as genes that can be used for visualization in vivo. Demidyuk and colleagues provide the methods to create and evaluate the cytotoxic action of a bicistronic plasmid expressing the yeast cytosine deaminase/uracil phosphoribosyltransferase fusion protein and the hepatitis A virus 3C protease (Chapter 11).

Suicide gene therapy can result in the elimination of cancer cells within a tumor beyond those cells that actually express the transgene, in a process termed the "bystander effect." This is achieved by the spread (via gap junctions) of cytotoxic anti-metabolites produced by the transgene to neighboring cells that may not have been transfected or transduced. Neschadim and Medin have developed a suicide gene therapy system that mediates a highly effective bystander effect, based on a variant of human deoxycytidine kinase that can phosphorylate the unusual nucleoside analogs bromovinyl deoxyuridine and L-deoxythymidine, which they describe in Chapter 12. Hirschberg and colleagues describe a modified form of photodynamic therapy that enhances the delivery of the cytosine deaminase gene into tumor cells and the cytotoxic effect of the locally produced 5-FU (Chapter 13). Finally, in Chapter 14, we provide the methods for TransfeX-mediated transfection of the plasmid pNGVL1-*tk* encoding HSV-tk under the control of the CMV promoter into HeLa cervical carcinoma, HSC-3 and H357 oral squamous cell carcinoma, and FaDu pharyngeal carcinoma cell lines, and the resulting cytotoxicity upon administration of ganciclovir.

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I would like to dedicate this volume to the memory of my Ph.D. advisor, Professor Shinpei Ohki at the State University of New York at Buffalo (now University at Buffalo).

San Francisco, CA, USA ***Nejat Düzgüneş***

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