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The goal of this proposal is to attempt a novel strategy for the use of viruses in the delivery of anti-cancer genes to prostate cancer cells. Current methods involving the use of viruses to deliver therapeutic genes for the treatment of any disease are hindered by the inability to target viral agents specifically to diseased tissue or organs. Recently, a protein marker has been characterized on the surface of prostate cancer cells. This marker, called the prostate-specific membrane antigen (PSMA), is readily detectable on prostate cancer cells from a large percentage of patients with prostate cancer. The abundance of this marker on cancerous prostate cells allows such cells to be detected and discriminated from both normal prostate cells and the cells of other bodily tissues. We have sought to analyze the possibility of utilizing the PSMA on the surface of prostate cancer cells as a way to target viral gene therapy agents specifically to tumors of the prostate.

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Mark W Pandolfi 7/24/00

PI - Signature Date

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Introduction

The goal of this proposal is to attempt a novel strategy for the use of viruses in the delivery of anti-cancer genes to prostate cancer cells. Current methods involving the use of viruses to deliver therapeutic genes for the treatment of any disease are hindered by the inability to target viral agents specifically to diseased tissue or organs. Recently, a protein marker has been characterized on the surface of prostate cancer cells. This marker, called the prostate-specific membrane antigen (PSMA), is readily detectable on prostate cancer cells from a large percentage of patients with prostate cancer. The abundance of this marker on cancerous prostate cells allows such cells to be detected and discriminated from both normal prostate cells and the cells of other bodily tissues. We have sought to analyze the possibility of utilizing the PSMA on the surface of prostate cancer cells as a way to target viral gene therapy agents specifically to tumors of the prostate.

Annual Summary

Over the course of the first year of this fellowship award, several accomplishments have been made toward the successful conclusion of the proposed work. Along with this work, there have been instances where certain aspects of the proposed work have been found to be difficult or inefficient. In these cases, alternative strategies have been investigated and developed. While these strategies are described as "alternative", contribution toward the goal of this work has been rigidly maintained in the implementation of these modifications in the origingal protocols.

Specific Aim I in the "Statement of Work" proposed the modification of the surface of retroviral particles such that antibodies to the prostate-specific membrane antigen (PSMA) could be stably attached. The proposed strategy sought to utilize biotinylated virions and biotinylated antibodies to PSMA which would be anchored to one another by the utilization of streptavidin as a bridging molecule.

The biotinylation of viral particles has been accomplished and analyzed. Through the use of Sulfo-NHS-LC-biotin, at a wide range of concentrations, we have successfully determined the conditions under which the successful biotinylation of retroviral particles can be achieved with minimal effect on biological activity (infectivity) of the viral particles. These experiments have been completed with retroviral gene delivery agents bearing either the envelope glycoprotein from the spleen necrosis virus (SNV) and the envelope glycoprotein from amphotropic murine leukemia virus (A-MLV). Viral particles bearing either of these envelope glycoproteins appear to behave identically to the biotinylation of their outer surface. It is also clear that over-biotinylation of these retroviral particles results in the destruction of their biological function. Biotinylation of the outer surface of retroviral particles was confirmed by demonstration of the ability to remove biotinylated retrivirus particles from solution by use of streptavidin-coated solid supports.

Subsequent to the biotinylation of retroviral particles, we have accomplished the attachment of streptavidin to the surface of retroviral particles. As proposed in the Statement of Work, this has been achieved by first biotinylating the surface of retroviral particles, as described above. After biotinylation of the viruses, the excess, non-virion-associated biotinylation reagent was removed by ultrafiltration. These modified retrovirus particles were then exposed to excess streptavidin. These viruses were analyzed for infectious titer and were found to possess infectivities equivalent to that of viruses which had been only biotinylated. However, we have found that the removal of excess biotinylation reagent by repeated rounds of ultrafiltration has resulted in a lowering of viral titer. In light of this, we have sought alternative methods for the attachment of streptavidin to the outer surface of retroviral particles.

Another method which we have investigated for the attachment of streptavidin to the surface of retroviral particles has involved the use of streptavidin-hydrazide derivatives. Streptavidin-hydrazide, a commerically available, chemically modified version of streptavidin, contains numerous hydrazino groups. The presence of such chemical groups allows for this streptavidin to be covalently attached to the aldehyde groups of oxidized galactosyl residues within carbohydrates. To attach streptavidin hydrazide to the surface of retroviral particles, we have first treated retorviral particles with the either sodium-meta-periodate or galactose oxidase and neuraminidase in combination. These treatments assured the oxidation of galactosyl residues in carbohydrate chains that are virion associated. We have found that sodium-meta-periodate-mediated oxidation of retrovirus particles is most likely too destructive, and results in an aboliton of biological activity of the retroviral particles. However, the use of the enzymes galactose oxidase and neuraminidase has performed successfully in the oxidation of the surface of retroviral particles such that the infectivity of treated particles is not substantially affected. The subsequent addition of streptavidin-hydrazide to such oxidized retroviral particles has been accomplished and verified. Verification of the association of streptavidin with retroviral particles has been achieved by demonstration of the ability to remove such treated particles from solution by use of a biotinylated solid-support.

We have recently investigated the possibility of using the streptavidin-hydrazide derivative for the construction of covalent streptavidin-anti-PSMA fusions. In so doing, existing monoclonal antibodies against PSMA would not need to be biotinylated prior to the attachment of such antibodies to the surface of biotinylated retroviral particles. Instead, purified complexes of streptavidin-hydrazide-anti-PSMA could simply be added to biotinylated particles prior to their use in targeting experiments. It is also quite possible that such anti-PSMA-streptavidin chemical fusions could be useful as effective detection reagents for PSMA, either in vivo or in vitro, even in the absence of their use as viral-targeting devices.

Simultaneous to the work described above, we have also accomplished the successful construction of a binary system of cell lines which either over-express, or do not express the prostate-specific membrane antigen. We have obtained a eukaryotic expression vector which has been demonstrated to allow for the over-expression of PSMA in cells to which it has been transfected. This vector also possesses the gene for resistance to neomycin, which allows for the selection of cells which have stably incorporated this plasmid into their genome. Utilizing this plasmid, we have created a pair of D17 cells which express either no detectable PSMA, or which over-express PSMA, as compared to the level of expression of PSMA in LNCaP cells. Having successfully constructed this binary system of cell lines for the expression of PSMA, we are now well-positioned for a rigorous analysis of any PSMA-specific retroviral particles which are constructed.

Key Accomplishments

- The construction of biotinylated retroviral gene delivery agents; demonstration of the amounts of biotin which result in maximal biotin incorporation with minimal effects on virion infectivity

- The attachment of streptavidin to the surface of retroviral gene delivery agents; accomplished in either of two ways: by the addition of streptavidin to biotinylated and purified viral particles, or by the use of streptavidin hydrazide

- Demonstration of the use of hydrazide-modified streptavidin as a means of attaching streptavidin molecules to the surfaces of retroviral particles

- The construction of streptavidin-hydrazide-anti-PSMA fusions by chemical means which are covalent and do not involve biotin-streptavidin interactions

- The construction of a binary system of cell lines which either over-express or do not express PSMA, for the anaylsis of any PSMA-specific targeting system