Review

Using viral genomics to develop viral gene products as a novel class of drugs to treat human ailments

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Abstract

A novel strategy for drug discovery and advancement has been developed by exploiting viral genomics. By manipulating cell function through the therapeutic administration of specific gene products from viruses, an important new class of therapeutics could be developed for the treatments of major human diseases. As it may turn out in the human genome arena, the groups which can establish a stronghold in the field would be in the best position to exploit this new drug development strategy.

Introduction

Throughout history, man has been plagued by diseases and ailments caused by pathogens including viruses. Accordingly, these pathogens have been targets for vaccine and drug development. The history of vaccine use dates back to ancient times. In the 7th century, some Indian Buddhists drank snake venom to protect themselves from snakebites. During the 9th century in China, *The Correct Treatment of Small Pox* was written by a Buddhist nun, who recommended a mixture of ground dried smallpox scabs and herbs to be blown into the nostril of children (Plotkin 1988). Today, vaccines have protected hundreds of millions of people from smallpox, poliomyelitis, measles, mumps, rubella, yellow fever, pertussis, hepatitis A and B, and chickenpox, as well as others. Still, whether they are common cold viruses or more formidable HIV-1 or Hepatitis C viruses, mankind's battle with these pathogens has been and will continue to be a constant challenge.

A major impediment for developing effective vaccines to the remaining viruses is that they are very efficient at evading and manipulating the human immune systems. Viruses have honed their ability to effectively and efficiently commandeer and control host cellular machinery to carry out their life cycle. Viruses are constructed from small genomes and must exploit the molecular machinery of the host cell for their propagation and other functions. These viral invaders have evolved over time to develop a system of potent protein armaments to evade and thwart immune and inflammatory responses mounted by the host immune system. Once they infect host cells, viruses engineer the cells to manufacture specific viral proteins or gene products, which they use as additional functional weapons to control host cell tasks. These well-honed tools are then used to enhance the conditions for their own survival and proliferation.

As this historic battle of man versus virus continues, a novel strategy has been developed whose goal is to harness and exploit pathogens' own tools – that is their specific gene products – to develop a new class of drugs and therapeutics. Rather than viewing viruses and other pathogens only as targets for pharmaceutical and vaccine development, the new approach aims to utilize the pathogens' specific gene products as potential drugs to address unmet medial needs.

Strategy of exploiting viral genomes as potential drugs

This new strategy is based on the premise that specific viral proteins, even when they are separated from their associated viruses, retain their potent ability to control and regulate important body functions in the absence of other disease causing parts of the virus. More specifically, these viral proteins with specific functions *in vivo* could be identified and exploited as potential drugs. By manipulating cell function through the therapeutic administration of specific gene products from pathogens such as viruses, an important new class of therapeutics could be developed for the treatments of major human diseases, including cancer, autoimmune diseases, and cardiovascular disorders.

There is a strong rationale for this approach. Host genes have evolved to fit carefully into the overall scheme of host biology. They have been selected not to harm the host through mechanisms, which would include some tolerance for abnormal expression and regulation. In these cases, only the culmination of the host gene and time will result in a significant effect manifested on the host. The pathogen genes have been selected for the opposite effect. Pathogens try to carry as little genetic material as possible. Viruses are the masters of this strategy. Yet even with so little baggage, in many cases, they are completely dependent on a single gene to drastically alter and control the cell biology of the host. Evolution has selected for complete potency and penetrance of the specific gene product. Accordingly adapting viral gene products to modulate human disease takes advantage of this significant and complete biological potency in humans and animals.

Development of HIV-1 Vpr gene products as novel drugs

In this regard, one specific example of viral protein drug targets for this strategy is a 96 amino acid HIV-1 Vpr (**v**iral **p**rotein **r**) (Haseltine 1991, Levy *et al.* 1993). As an important accessory protein for HIV-1, one of Vpr's functions is to keep the cell from going through normal cell division (Figure 1) (Emerman 1996, Levy 1995b). For the benefit of the virus, delaying cells at this point of the cell cycle allows HIV-1 to increase virus production in the cell (Levy 1994, 1995a). This property of Vpr can be exploited to develop treatments for a variety of ailments, including cancer. Cancer is a group of diseases characterized by

uncontrolled growth and the proliferation of abnormal cells. In this regard, Vpr prevents the proliferation of cells by causing them to accumulate in the G2 phase of the cell cycle and subsequently to lead to killing of the cells by apoptosis (Ayyavoo 1997b, Jowett 1995, Stewart 1997). Apoptosis is cell's natural pre-programmed death that occurs in a timely and non-traumatic fashion as part of the body's natural development and maintenance of healthy tissues and organs. In fact, a major way cancer cells proliferate out of control in the body is to suppress the cells' ability to induce apoptosis.

It is estimated that defects in apoptosis are estimated to directly or indirectly contribute to the pathogenesis of nearly 70% of all human illnesses, including cancer and autoimmune diseases. By engineering apoptosis *in vivo*, pharmaceutical products derived from Vpr proteins, which modulate these cellular functions, may have important clinical relevance in oncology and other medical areas. Cancer ranks second to cardiovascular disease as a cause of death in the United States and accounts for about 25% of all deaths. According to the American Cancer Society (ACS), approximately 1.2 million cancer cases were diagnosed in 2000, of which approximately 50% of newly diagnosed cancers are breast, colon, lung or prostate. This number is likely to go up as newer diagnostic and imaging technologies provide for earlier diagnosis. ACS estimates about 550 000 cancer-related deaths will also occur this year. The economic impact of cancer is enormous. The total cost of cancer is estimated to be $107 billion in the US alone. These costs include $37 billion of direct medical cost and $70 billion in indirect morbidity and mortality costs. Effective treatment of cancer has eluded medical practitioners as most treatments either indiscriminately destroy healthy cells or trigger adverse reactions, which may themselves be harmful.

Application of engineered Vpr protein (with no association to HIV itself) has been shown to prevent tumor cell growth and lead to killing of the cells by apoptosis in both human tumor cells in culture and in tumor models in mice (Figure 2). Vpr proteins have been shown to be effective against a variety of established human tumor cells lines derived from lung, breast, prostate, brain, and other sources (Table 1) (Mahalingam 1997, Shostak 1999, Stewart 1999). Vpr has been shown to be effective in cancer cells, which have disabled both the p53 regulatory system and the Fas-mediated system. Furthermore, the specific sequences or fragments of the Vpr peptides have also

Fig. 1. HIV-1 genome. Vpr is an important accessory protein for HIV-1.

Fig. 2. (A) Vpr helps to maximize HIV production by controlling the host cell cycle and function. (B) Vpr-based Rx stops tumor cells from growing/dividing and kills them via apoptosis.

shown to have active therapeutic properties and are a target for further development (Ayyavoo 1997a).

In addition to cancer indications, Vpr has been shown to directly mediate T-cell immune responses. Broad disease category of autoimmune diseases (arthritis, multiple sclerosis, etc.) is a set of T-cell me-diated immune diseases, which could be particularly responsive to Vpr-based therapies. In an autoimmune disease, the body's own defense mechanism whose job is to protect the body against foreign pathogens begins to attack the body itself. In these cases one or both

Table 1. Tumors cells responsive to Vpr treatment.

| Cell lines	Cancer origin	Phenotype	Ref.
SW480	Human colon cancer	p53 mutant	Mahalingam 1997
T24	Human bladder cancer	ras H v12	
MCF-7	Human breast cancer	ras K 12	
D324	Human medullablastoma	p53 mutant	
R175 REF p53m	Rat fibroblast	p53 mutant transfected	
HCT116	Human colorectal cancer	p53 wildtype	Shostak 1999
Saos-2	Human osteocarcinoma	p53 mutant	
HeLa	Human cervical carcinoma	p53 mutant	
SV80	Transformed human fibroblast	p53 mutant	
Weri	Human retinoblastoma	p53 wildtype	
SiHa	Human cervical carcinoma	p53 mutant	
SW480	Human colon adenocarcinoma	p53 mutant	Stewart 1999
HT1080. ATCC	Human fibrocarcinoma	p53 wildtype	
HT1080.6TG	Human fibrocarcinoma	p53 mutant	
SupT1	Human T cell lymphoma	unknown	
LNCap	Human prostate carcinoma	p53 wildtype	
HCT116	Human colon carcinoma	hMLH	
XP 12BESV	*Xeroderma pigmentosum*, complementation group A	XPA	
HeLa	Human cervical carcinoma	p53 mutant	
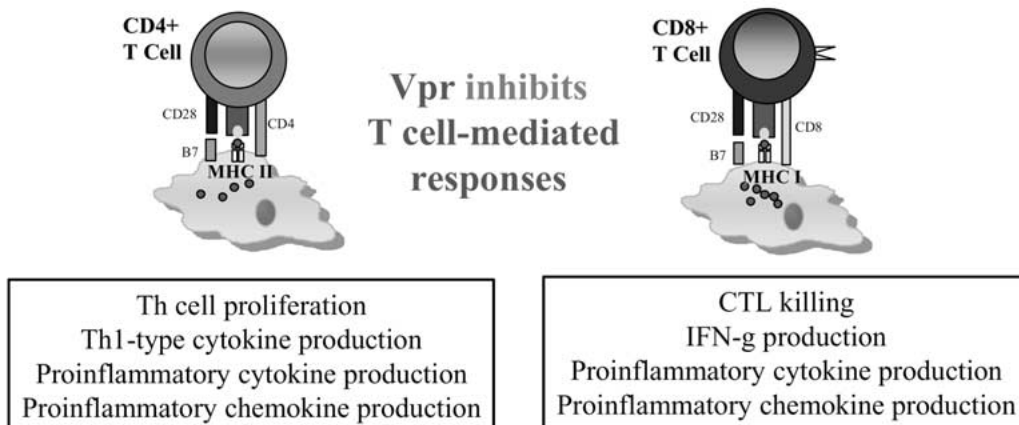
Fig. 3. Vpr inhibits T cell-mediated immune responses.

of immune 'soldiers' – T or B cells – turn against the body, resulting in painful suffering and even death.

In this respect, Vpr has been shown to selectively shut down T-cell activities upon activation. These activities included dramatic reduction of T cell proliferation (both Th1-type CD4+ T cells and CD8+ killer T cells) and abrogation of proinflammatory cytokine and chemokine production (see Figure 3) (Ayyavoo 1997b, Muthumani 2000). Importantly, Vpr did not affect resting cells dramatically. This suggests an important window for drug development with Vpr-based reagents. These properties could be used to develop drugs for a variety of autoimmune diseases including arthritis and psoriasis.

Drugs targeting Vpr's cellular targets

A protein named 'mov34/hVIP' was identified as a target for Vpr in the cells (Mahalingam 1998). Inhibition

or blockage of mov34 in tumor cell lines is 100% effective at preventing human tumor cell growth in the laboratory. Successful treatment of prostate, breast, brain, leukemia, bladder, and liver tumor cell lines has been achieved. This discovery illustrates the value of the mov34 gene product as a target for drug development. In addition to tumor cell lines, activated T cells also express mov34, supporting it as a target for the development of autoimmune disease. In addition, understanding the mechanism and molecular interactions between Vpr and mov34 presents additional opportunities to develop small molecule drugs to regulate various specific immune functions in the body.

Potential hurdles for developing drugs through viral genomics

Development of viral proteins as drugs is not immune to potential hurdles. One major issue is that viral proteins could elicit immune responses, which could neutralize their effectiveness. Potential immune responses induced in humans from the introduction of foreign viral proteins in general could result in unwanted inflammatory reactions as well as in reduction of drug efficacy. Another potential issue is the delivery of Vpr-based drug through the cell membrane, the cytoplasm, and the nuclear membrane to the nucleus.

In this regard, not all viral proteins are created equal. Although Vpr is a viral protein, it is not a typical viral protein. In fact, one of the ways HIV-1 evades the human immune system so well is through the production of Vpr. Vpr has been shown to be extremely effective in shutting down T cell-mediated arm of the immune responses (Th1-type CD4+ T cell responses as well as CD8+ CTL responses). Although Vpr has not been shown to down-regulate the antibody-mediated responses, Vpr is not known to have strong antibody epitopes, even in HIV-1 infected patients. Even though it is estimated that there are about 5–50 μg of HIV-derived Vpr in the body (each virus contains 2400 copies of Vpr) in HIV-1 infected patients, there have been no reported incidents of acute toxicity to Vpr or HIV-1 infection as a whole at early time points in infection. Furthermore, additional measures could be taken to mitigate potential issues of immune responses by selecting more acute clinical targets and by utilizing more focused local delivery methods.

Targeting the drug to the specific site of interest is not a problem unique to VGX drugs. One of the biggest problems of currently available chemotherapeutic drugs is systemic and non-selective toxicity. Vpr is a soluble protein and has been shown to enter the target tumor cells (or all cells for that matter) through cell membrane and traffic to nucleus. In fact Vpr has one of the most potent nuclear-localization signals within its structure. Delivery of Vpr into the cell has been shown to be a very effective and efficient process. In addition, Vpr has been shown to be selective in its effect. Vpr is more efficacious in more actively dividing cells (i.e., Tumor cells) versus the non-dividing cells (normal cells) (Ayyavoo 1997b, Stewart 1999). This selectivity has potential advantages over the current chemotherapies.

The path forward

By exploiting viral genomics, a new class of novel drugs to treat human ailments could be developed. The efforts needed in these developmental programs include a successful preclinical toxicology analysis of these drug compounds and the subsequent clinical trials to test their effects in humans. To provide an accelerated clinical review path, it may be preferable to target initial, strategically important markets (such as cancer), which do not currently have adequate therapies.

As discerning potentially useful viral gene products from a countless of number of viral proteins would not be a simple task, systematic and concerted efforts utilizing viral functional genomics and proteomics methods are needed to expand the potential of this strategy to expand the portfolio of useful viral protein candidates. As it may turn out in the human genome arena, the groups which can establish a stronghold in the field would be in the best position to exploit this new drug development strategy.

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