Adenovirus Expressing RIZ1 in Tumor Suppressor Gene Therapy of Microsatellite-unstable Colorectal Cancers

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Abstract

Viral vector-mediated delivery of tumor suppressor genes represents a promising strategy of cancer therapy. Several best-studied tumor suppressor genes, such as p53 and retinoblastoma (Rb), have been evaluated for gene therapy of tumors that carry mutations in these genes. However, these genes may not be applicable to microsatellite instability positive (MSI(+)) tumors because they are rarely mutated in these tumors. The Rb-interacting zinc finger gene RIZ1 is commonly mutated in MSI(+) colorectal, gastric, and endometrial cancers and has demonstrated a capacity to induce cell cycle arrest and apoptosis. Here, we found that RIZ1 expression through adenovirus vectors suppressed growth of MSI(+) HCT116 colorectal xenograft tumors that carry RIZ1 mutations. Malignant cells in the established tumors were efficiently transduced by RIZ1 adenoviruses and underwent apoptosis in response to RIZ1 expression. In comparison, a recombinant p53 adenovirus did not induce apoptosis and tumor suppression. These results suggest that RIZ1 may be useful in gene therapy of MSI(+) colorectal cancers.

Introduction

Colorectal cancer is the third most common cancer in Western society (1). It is now commonly believed that all cancers result from the accumulation of genetic alterations in cellular cancer-causing genes. These alterations are thought to be driven by genetic instabilities. Two major genetic instability pathways have been recognized in colorectal cancers, chromosomal instability and microsatellite instability (2). The hallmarks of tumors of the chromosomal instability pathway are aneuploidy and loss of heterozygosity. In contrast, tumors of the microsatellite instability pathway are usually diploid and show massive instability in simple repeated sequences or microsatellites. In addition, epigenetic events can also facilitate genetic damage, as illustrated by the increased mutagenicity of 5-methylcytosine and the silencing of the MLH1 mismatch repair gene by DNA methylation in colorectal tumors (3).

MSI(+) tumors are caused by defects in mismatch repair system (4–6). MSI has been detected in hereditary nonpolyposis colorectal, gastric, endometrial, and pancreatic cancers (7). The mechanism of tumorigenesis of MSI(+) tumors is thought to involve frameshift mutations of microsatellite repeats within coding regions of affected target genes, the inactivation of which directly contributes to tumor development (8).

The gene RIZ1 is a candidate tumor suppressor gene belonging to the PR or SET domain family of chromosomal regulators involved in chromatin-mediated gene activation and silencing (9, 10). The PR/SET domain family plays an important role in human cancers as evidenced by genetic alterations of several members of this family (11). The function of the PR/SET domain is not well understood. The PR domain of RIZ appears to be a protein-binding interface and can interact with a motif present in the COOH region of RIZ (10).

RIZ1 also plays an important role in MSI(+) tumors, as suggested by the frequent frameshift mutations in MSI(+) colorectal, gastric, endometrial, and pancreatic cancers (16–18). The mutations are located at two polyadenosine tracks within the coding region of RIZ: one (A)8 track at coding nucleotide position 4273–4280, and one (A)9 track at 4462–4471 in exon 8. These mutations generate truncated RIZ1/2 proteins lacking the COOH-terminal PR-binding motif and are expected to have serious deleterious effects on the PR domain-specific function of RIZ1. These frameshift mutations appear positively selected because similar mutations are rare in other randomly selected genes with (A)8 tracks.

A large number of studies have been performed to evaluate the potential of tumor suppressor gene delivery in cancer therapy. These studies generally involve familial tumor suppressor genes such as p53 and Rb and MSI(−) tumors that carry mutations in these genes. Adenovirus-mediated p53 gene delivery is being tested in human clinical trials to treat non-small cell carcinoma and head and neck cancers (19). Relatively little is done on MSI(+) tumors. That these tumors do not carry mutations in p53 and Rb necessarily precludes the use of these genes. In view of the fact that RIZ1 is commonly mutated in MSI(+) tumors and has demonstrated growth-inhibitory activities, we examined whether RIZ1 may be useful in gene therapy of MSI(+) tumors. Our results show that RIZ1 expression mediated by adenovirus vectors suppressed growth of RIZ1-mutated HCT116 colorectal xenografts.

Materials and Methods

Cell Lines and Adenovirus Infections in Vitro. The RIZ1-mutated human colorectal carcinoma cell line HCT116 was purchased from American Type Culture Collection. Cells were cultured in DMEM with 10% FCS. Adenovirus lacking an insert (Adnull; a kind gift of Prem Seth, National Cancer Institute, Bethesda, MD) or expressing either RIZ1 (AdRIZ1) or p53 (Adp53) were amplified and titered in 293 cells (14).

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The abbreviations used are: MSI(+), microsatellite instability positive; Rb, retinoblastoma; RIZ, Rb-interacting zinc finger; PR, PR Domains-BF1-RIZ1 homology; SET, Su(var)3-9, Enhancer-of-zeste, Trithorax homology.
Tumors were allowed to grow in vivo for 6 days when they reached an average size of 0.5 cm in diameter. Prior to therapy, the animals were randomized and regrouped by tumor size (5–11 mice/group). Intratumoral and peritumoral injection of 100 μl of PBS, Adnull, Adp53, or AdRIZ1 virus suspension (for all viruses, 8 × 10^10 particles of virus/dose) were then administered on every other day for total of four doses to mice bearing tumors. Tumor sizes were measured two to three times a week. Tumor volume was calculated as a^2b^2 × 0.5, where a is the length and b is the width of the tumor in millimeters. Tumor volumes for different treatment groups on each day were compared by Student’s t test.

Results and Discussion

To determine the efficacy of RIZ1 in gene therapy of MSI(+) tumors in vivo, we examined whether the recombinant RIZ1 adenoviruses could inhibit growth of established solid tumors in vivo. As a control for genes not mutated in MSI(+) cancers, we also studied the effects of recombinant p53 adenoviruses. We selected the MSI(+) HCT116 colorectal cancer cell line for our studies because it carries homozygously mutated RIZ1 and wild-type p53 and is susceptible to RIZ1-induced cell cycle arrest and apoptosis in vitro (16). We injected HCT116 tumor cells s.c. into nude mice. Mice bearing established HCT116 tumors received intratumoral and peritumoral injections of either PBS alone, Adnull, AdRIZ1, or Adp53 viruses in suspension, respectively. In the mice treated with buffer alone, the Adnull virus, or the Adp53 virus, tumors continued to grow aggressively (Fig. 1). In contrast, all tumors that received four doses of AdRIZ1 virus grew significantly slower.

We next examined transduction of tumor cells by the AdRIZ1 and Adp53 viruses and the effects of RIZ1 or p53 expression on tumor cell apoptosis in vivo. We injected HCT116 tumor cells into nude mice and allowed the xenografts to grow for 12 days. Virus suspension was injected intratumorally into the established tumors. At day 2 after
injection of the viruses, the tumors were excised and processed for immunohistochemical staining of RIZ1, p53, or fragmented DNA in paraffin-embedded tissue sections. As shown in Fig. 2, the majority of tumor cells surrounding the injection sites showed strong RIZ1 or p53 nuclear staining in AdRIZ1 virus- or Adp53 virus-injected tumors, respectively. In addition, tumors injected with AdRIZ1 but not Adp53, Adnull, or buffer alone showed Apoptag staining, indicating that apoptosis occurred in RIZ1-expressing cells. Similar results were also observed for large xenograft tumors that were injected with virus at 1 month after tumor cell inoculation (data not shown).

Our preclinical studies here demonstrate that treatment of MSI(+) colorectal cancer xenografts in nude mice by a recombinant adenovirus vector, AdRIZ1, suppressed the growth of the treated tumors. Consistent with previous observations in vitro (16), virus-transduced cells of the HCT116 tumor xenograft underwent apoptosis in response to RIZ1 expression. As might be expected, p53, which is not involved in MSI(+) cancers, failed to induce tumor suppression and apoptosis in the same experimental settings. Although p53 shows great promise in MSI(+) cancers, failed to induce tumor suppression and apoptosis in the proximal colon [see comments]. Science (Washington DC), 260: 816–819, 1993.

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Two types of RIZ1 inactivation have been commonly observed in human cancers, RIZ1 gene silencing and frameshift mutations. Although only gene silencing is found in MSI(−) tumors, both have been found in MSI(+) tumors (16). RIZ1 inactivation, through either gene silencing or frameshift mutations, may occur in at least 30–50% of MSI(+) cancers of the colorectum, stomach, and endometrium (16–18). In addition, RIZ1 is a transcription factor and may share similar mode of actions with the best-studied tumor suppressors, such as Rb and p53. In turn, knowledge gained from gene therapy studies on Rb and p53 may be more directly applied to RIZ1. These observations, together with the present preclinical study, suggests that RIZ1 merits serious consideration for application in gene therapy of MSI(+) cancers.

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References


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