Claims

1. A recombinant adenovirus comprising

a first nucleotide sequence inserted in an IX-E2 insertion site, wherein the IX-E2 insertion site is located between the stop codon of adenovirus IX gene and the stop codon of adenovirus IVa2 gene;

a second nucleotide sequence inserted in an L5-E4 insertion site, wherein the L5-E4 insertion site is located between the stop codon of adenovirus fiber gene and the stop codon of ORF6 or ORF6/7 of adenovirus E4 gene;

or both the first nucleotide sequence inserted in the IX-E2 insertion site and the second nucleotide sequence inserted in the L5-E4 insertion site,

wherein the first nucleotide sequence inserted in the IX-E2 insertion site comprises, in a 5' to 3' orientation:

(i) a first IX-E2 polyadenylation signal;

(iii) a IX-E2 promoter;

(iv) a first transgene;

(v) a third IX-E2polyadenylation signal; and

(vi) a fourth IX-E2 polyadenylation signal;

wherein the first IX-E2 polyadenylation signal is the polyadenylation signal of the IX gene, the third IX-E2 polyadenylation signal is the polyadenylation signal of the first transgene, and the fourth IX-E2 polyadenylation signal is the polyadenylation signal of the adenovirus IVa2 gene; and

wherein the second nucleotide sequence inserted in an L5-E4 insertion site comprises, in a 5' to 3' orientation:

(i) a first L5-E4 polyadenylation signal;

(ii) an second L5-E4 polyadenylation signal;

(iii) the L5-E4 promoter;

(iv) the second transgene;

(v) a third L5-E4 polyadenylation signal; and

(vi) a fourth L5-E4 polyadenylation signal;

wherein the second L5-E4 polyadenylation signal is in the opposite transcriptional direction of the first L5-E4 polyadenylation signal, and wherein the first L5-E4 polyadenylation signal is the polyadenylation signal of the fiber (L5) gene, the third L5-E4 polyadenylation

signal is the polyadenylation signal of the second transgene, and the fourth L5-E4 polyadenylation signal is the polyadenylation signal of the ORF6 or ORF6/7 of the adenovirus E4 gene.

2. The recombinant adenovirus of claim 1, wherein the first nucleotide sequence is inserted between nucleotides corresponding to 4029 and 4093 of the Ad5 genome (SEQ ID NO: 1) or between nucleotides corresponding to 3899 and 3970 of the Ad35 genome (SEQ ID NO: 41).

3. The recombinant adenovirus of claim 2, wherein the second nucleotide sequence is inserted between nucleotides corresponding to 32785 to 32916 of the Ad5 genome (SEQ ID NO: 1) or between nucleotides corresponding to 31799 and 31821 of the Ad35 genome (SEQ ID NO: 41).

4. The recombinant adenovirus of claim 1, wherein the first nucleotide sequence further comprises a second IX-E2 polyadenylation signal between the first IX-E2 polyadenylation signal and the IX-E2 promoter and the second polyadenylation signal is in the opposite transcriptional direction of the first polyadenylation signal.

5. The recombinant adenovirus of any one of claims 1-4, wherein the recombinant adenovirus further comprises a nucleotide sequence inserted in an E1b-19K insertion site, an E3 insertion site, or an E4 insertion site.

6. The recombinant adenovirus of any one of claims 1-5, wherein the recombinant adenovirus selectively replicates in a hyperproliferative cell.

7. The recombinant adenovirus of any one of claims 1-6, wherein the recombinant adenovirus selectively expresses the first transgene, the second transgene, or both the first transgene and the second transgene in a hyperproliferative cell.

8. Use of the recombinant adenovirus of any one of claims 1-7 in the manufacture of a medicament for inhibiting tumor growth.

9. A pharmaceutical composition comprising the recombinant adenovirus of any one of claims 1-7 and at least one pharmaceutically acceptable carrier or diluent.

10. A formulation for adenoviruses comprising:

(a) the recombinant adenovirus of any one of claims 1-7;

(b) at least one buffer;

(c) at least one tonicity modifier;

(d) at least one sugar or at least one stabilizing agent, or both; and

wherein the formulation has a pH ranging between about 7.0 and about 9.0.