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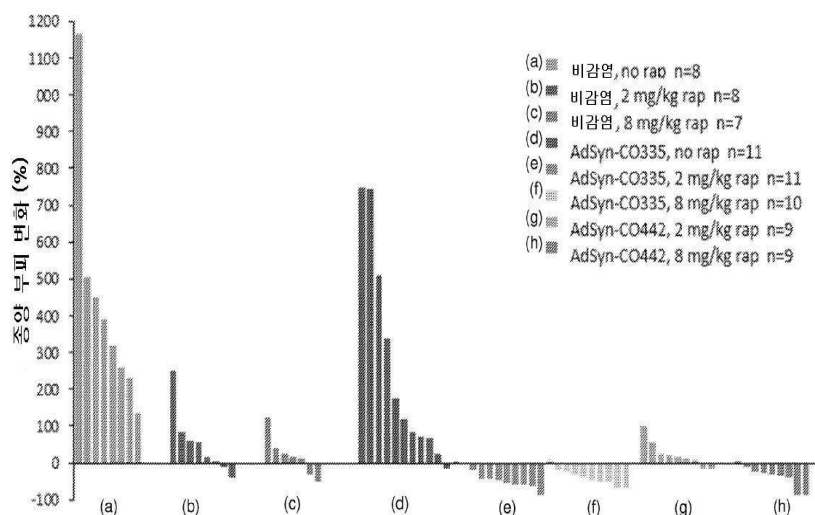
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(54) 발명의 명칭 **종양 살상 바이러스 및 이의 사용방법**

(57) 요약

E2F 비조절 종양 세포에서 선별적으로 복제하는 재조합 아데노바이러스를 개시한다. 상기 재조합 아데노바이러스는 종양세포와 비교하여 정상세포에서 복제결함을 나타내는, 변형된 E1A 단백질, 변형되거나 결실된 E4orf1 단백질, 변형되거나 결실된 E4orf6/7 단백질, 또는 이의 임의의 조합을 암호화하는 게놈을 가지며, 종양세포와 비교하여 정상세포에서 복제결함을 나타낸다. 경우에 따라, 재조합 아데노바이러스 게놈은 재조합 아데노바이러스를 특정 세포 유형으로 표적화하고, 간에서 바이러스를 비표적화시키고, 간에서 바이러스 복제를 억제하고/하거나 이미 존재하는 중화 항체를 회피하는 추가적인 변형을 암호화한다.

대표도 - 도30



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명세서

청구범위

청구항 1

변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 변형된 또는 결실된 E4orf6/7 단백질, 또는 이들의 임의의 조합을 암호화하는 게놈을 가지며, 종양 세포와 비교하여 정상 세포에서 복제 결함을 갖는, 재조합 아데노바이러스.

청구항 2

제1항에 있어서, 상기 게놈은 변형된 E1A 단백질, 및 변형된 또는 결실된 E4orf1 단백질을 암호화하는, 재조합 아데노바이러스.

청구항 3

제1항에 있어서, 상기 게놈은 변형된 E1A 단백질 및 결실된 E4orf6/7 단백질을 암호화하는, 재조합 아데노바이러스.

청구항 4

제1항에 있어서, 상기 게놈은 변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질을 암호화하는, 재조합 아데노바이러스.

청구항 5

제1항 내지 제4항 중 어느 한 항에 있어서, 상기 변형된 E1A 단백질은 하기를 포함하는, 재조합 아데노바이러스:

LXCXE 모티프의 결실;

2 내지 11 잔기의 결실;

C124G 치환;

Y47H 치환;

Y47H 치환 및 C124G 치환; 또는

Y47H 치환, C124G 치환 및 2 내지 11 잔기의 결실.

청구항 6

제1항, 제2항, 제4항 및 제5항 중 어느 한 항에 있어서, 상기 변형된 E4orf1 단백질은 PDZ-결합 모티프의 결실을 포함하는, 재조합 아데노바이러스.

청구항 7

제1항에 있어서, 상기 게놈이 하기를 암호화하는, 재조합 아데노바이러스:

LXCXE 모티프의 결실을 포함하는 변형된 E1A 단백질 및 결실된 E4orf6/7 단백질;

LXCXE 모티프의 결실을 포함하는 변형된 E1A 단백질, PDZ-결합 모티프의 결실을 포함하는 변형된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질;

LXCXE 모티프의 결실을 포함하는 변형된 E1A 단백질, 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질;

C124G 치환을 포함하는 변형된 E1A 단백질 및 결실된 E4orf6/7 단백질;

C124G 치환을 포함하는 변형된 E1A 단백질, PDZ-결합 모티프의 결실을 포함하는 변형된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질;

C124G 치환을 포함하는 변형된 E1A 단백질, 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질;

2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질 및 결실된 E4orf6/7 단백질;

2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질, PDZ-결합 모티프의 결실을 포함하는 변형된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질;

2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질, 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질;

Y47H 치환 및 C124G 치환을 포함하는 변형된 E1A 단백질, 및 결실된 E4orf6/7 단백질;

Y47H 치환 및 C124G 치환을 포함하는 변형된 E1A 단백질, PDZ-결합 모티프의 결실을 포함하는 변형된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질;

Y47H 치환 및 C124G 치환을 포함하는 변형된 E1A 단백질, 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질;

Y47H 치환, C124G 치환 및 2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질, 및 결실된 E4orf6/7 단백질;

Y47H 치환, C124G 치환 및 2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질, PDZ-결합 모티프의 결실을 포함하는 변형된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질; 또는

Y47H 치환, C124G 치환 및 2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질, 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질.

청구항 8

제7항에 있어서, 상기 게놈이 서열번호 6 내지 8, 10 내지 12, 14, 15, 18 내지 20 및 22 내지 24중 어느 하나의 뉴클레오티드 서열을 포함하는, 재조합 아데노바이러스.

청구항 9

제1항 내지 제7항 중 어느 한 항에 있어서, 상기 게놈이 FK506 결합 단백질(FKBP)에 융합된 표적화 리간드, 및 야생형 FRB(FKBP-rapamycin binding) 단백질 또는 T2098L 치환을 포함하는 돌연변이 FRB 단백질에 융합된 아데노바이러스 섬유 단백질을 추가로 암호화하는, 재조합 아데노바이러스.

청구항 10

제9항에 있어서, 상기 표적화 리간드가 단일 도메인 항체인, 재조합 아데노바이러스.

청구항 11

제10항에 있어서, 상기 단일 도메인 항체가 EGFR에 특이적인, 재조합 아데노바이러스.

청구항 12

제1항 내지 제7항 및 제9항 내지 제11항 중 어느 한 항에 있어서, 상기 게놈이 E3-RID α /RID β 및 E3-14.7k 코딩서열의 결실을 포함하는, 재조합 아데노바이러스.

청구항 13

제9항 내지 제12항 중 어느 한 항에 있어서, 상기 게놈이 서열번호 25 또는 서열번호 26의 뉴클레오타이드 서열을 포함하는, 재조합 아데노바이러스.

청구항 14

제1항 내지 제7항 및 제9항 내지 제12항 중 어느 한 항에 있어서, 상기 게놈이 변형된 핵소 단백질질을 추가로 암호화하는, 재조합 아데노바이러스.

청구항 15

제14항에 있어서, 상기 변형된 핵소 단백질이 E451Q 치환을 포함하는, 재조합 아데노바이러스.

청구항 16

제14항 또는 제15항에 있어서, 상기 게놈이 서열번호 30, 서열번호 92 또는 서열번호 97의 뉴클레오타이드 서열을 포함하는, 재조합 아데노바이러스.

청구항 17

제1항 내지 제7항 중 어느 한 항에 있어서, 상기 게놈이 T2098L 치환을 포함하는 돌연변이 FRB 단백질에 융합된 아데노바이러스 섬유 단백질질을 추가로 암호화하는, 재조합 아데노바이러스.

청구항 18

제17항에 있어서, 상기 게놈이 E451Q 치환을 포함하는 변형된 핵소 단백질을 추가로 암호화하는, 재조합 아데노바이러스.

청구항 19

제17항 또는 제18항에 있어서, 상기 게놈이 서열번호 31의 뉴클레오타이드 서열을 포함하는, 재조합 아데노바이러스.

청구항 20

제1항 내지 제19항 중 어느 한 항에 있어서, 상기 게놈이 간 특이적인 마이크로 RNA를 위한 하나 또는 그 이상의 결합 부위를 추가로 포함하는, 재조합 아데노바이러스.

청구항 21

제20항에 있어서, 상기 간 특이적인 마이크로 RNA를 위한 하나 또는 그 이상의 결합 부위가 E1A의 3'-UTR에 위치하는, 재조합 아데노바이러스.

청구항 22

제20항 또는 제21항에 있어서, 상기 간 특이적인 마이크로 RNA가 miR-122인, 재조합 아데노바이러스.

청구항 23

제1항 내지 제22항 중 어느 한 항에 있어서, 상기 게놈이 키메라(chimeric) 섬유 단백질을 암호화하는, 재조합 아데노바이러스.

청구항 24

제23항에 있어서, 상기 키메라 섬유 단백질이 제1 아데노바이러스 혈청형으로부터의 섬유 샤프트(shaft) 및 제2 아데노바이러스 혈청형으로부터의 섬유 노브(knob)를 포함하는, 재조합 아데노바이러스.

청구항 25

제24항에 있어서, 상기 제1 아데노바이러스 혈청형이 Ad5이고, 제2 아데노바이러스 혈청형이 Ad3, Ad9, Ad11, Ad12, Ad34 또는 Ad37인, 재조합 아데노바이러스.

청구항 26

제25항에 있어서, 상기 제1 아데노바이러스 혈청형이 Ad5이고, 제2 아데노바이러스 혈청형이 Ad11, Ad34 또는 Ad37인, 재조합 아데노바이러스.

청구항 27

제26항에 있어서, 상기 게놈이 서열번호 82, 서열번호 83 또는 서열번호 84의 뉴클레오타이드 서열을 포함하는, 재조합 아데노바이러스.

청구항 28

제1항 내지 제27항 중 어느 한 항에 있어서, 상기 게놈이 캡시드가 교환된(capsid-swapped) 키메라 아데노바이러스를 암호화하는, 재조합 아데노바이러스.

청구항 29

제28항에 있어서, 상기 게놈의 E1, E3 및 E4 영역이 제1 아데노바이러스 혈청형으로부터 유래되고, 상기 게놈의 E2B, L1, L2, L3, E2A 및 L4 영역이 제2 아데노바이러스 혈청형으로부터 유래된, 재조합 아데노바이러스.

청구항 30

제29항에 있어서, 상기 제1 아데노바이러스 혈청형의 E1 영역이 제2 아데노바이러스 혈청형으로부터의 pIX 단백질을 암호화하도록 변형; 및/또는 상기 제1 아데노바이러스 혈청형의 E3 영역이 제2 아데노바이러스 혈청형으로

부터의 U엑손(Uexon) 및 섬유 단백질을 암호화하도록 변형된, 재조합 아데노바이러스.

청구항 31

제29항 또는 제30항에 있어서, 상기 제1 아데노바이러스 혈청형이 Ad5이고, 제2 아데노바이러스 혈청형이 Ad3, Ad9, Ad11 또는 Ad34인, 재조합 아데노바이러스.

청구항 32

제31항에 있어서, 상기 게놈이 서열번호 85, 서열번호 92, 서열번호 97, 서열번호 93, 서열번호 94, 서열번호 95, 또는 서열번호 96의 뉴클레오티드 서열을 포함하는, 재조합 아데노바이러스.

청구항 33

제1항 내지 제32항 중 어느 한 항에 있어서, 상기 게놈이 변형된 펜톤(penton) 단백질을 추가로 암호화하는, 재조합 아데노바이러스.

청구항 34

제33항에 있어서, 상기 변형된 펜톤 단백질이 인테그린-결합 RGD 모티프에서의 돌연변이를 포함하는, 재조합 아데노바이러스.

청구항 35

제34항에 있어서, 상기 게놈이 서열번호 86 또는 서열번호 87의 뉴클레오티드 서열을 포함하는, 재조합 아데노바이러스.

청구항 36

제1항 내지 제35항 중 어느 한 항에 있어서, 상기 게놈이 RGD 펩티드를 포함하도록 변형된 섬유 단백질을 추가로 암호화하는, 재조합 아데노바이러스.

청구항 37

제36항에 있어서, 상기 게놈이 서열번호 87의 뉴클레오티드 서열을 포함하는, 재조합 아데노바이러스.

청구항 38

제1항 내지 제37항 중 어느 한 항의 재조합 아데노바이러스 및 약학적으로 허용가능한 담체를 포함하는 조성물.

청구항 39

제1항 내지 제37항 중 어느 한 항의 재조합 아데노바이러스 또는 제38항의 조성물과 종양세포를 접촉시키는 것을 포함하는 종양세포 생존능을 저해하는 방법.

청구항 40

제39항에 있어서, 상기 방법이 시험관 내(*in vitro*) 방법인, 방법.

청구항 41

제39항에 있어서, 상기 방법이 생체 내(*in vivo*) 방법이고, 상기 종양세포를 접촉시키는 것이 상기 재조합 아데노바이러스 또는 조성물을 종양을 갖는 대상에게 투여하는 것을 포함하는, 방법.

청구항 42

제1항 내지 제37항 중 어느 한 항의 재조합 아데노바이러스 또는 제38항의 조성물의 치료학적 유효량을 대상에게 투여하여 대상에서의 종양의 진행을 저해하거나 종양의 부피를 감소시키는 것을 포함하는 대상의 종양 진행을 저해하거나 종양 부피를 감소시키는 방법.

청구항 43

제1항 내지 제37항 중 어느 한 항의 재조합 아데노바이러스 또는 제38항의 조성물의 치료학적 유효량을 대상에게 투여하여 대상에서의 암을 치료하는 것을 포함하는 대상의 암을 치료하는 방법.

청구항 44

하기를 대상에게 투여하는 것을 포함하는 대상의 종양 부피를 감소시키는 방법:

- (i) 제9항 내지 제16항 중 어느 한 항의 재조합 아데노바이러스의 치료학적 유효량; 및
- (ii) 라파마이신 또는 이의 유사체.

청구항 45

하기를 대상에게 투여하는 것을 포함하는 대상의 암을 치료하는 방법:

- (i) 제9항 내지 제16항 중 어느 한 항의 재조합 아데노바이러스의 치료학적 유효량; 및
- (ii) 라파마이신 또는 이의 유사체.

청구항 46

제44항 또는 제45항에 있어서, 상기 라파마이신 또는 라파마이신 유사체가 약 0.1 mg/kg 내지 약 2.0 mg/kg의 투여량으로 투여되는, 방법.

청구항 47

제44항 또는 제45항에 있어서, 상기 라파마이신 또는 라파마이신 유사체가 약 0.1 mg/kg 내지 약 0.5 mg/kg의 투여량으로 투여되는, 방법.

청구항 48

제44항 또는 제45항에 있어서, 상기 라파마이신 또는 라파마이신 유사체가 약 1 내지 약 15 mg/m²의 투여량으로

투여되는, 방법.

청구항 49

제44항 또는 제45항에 있어서, 상기 라파마이신 또는 라파마이신 유사체가 약 3 내지 약 10 ng/mL의 투여량으로 투여되는, 방법.

청구항 50

제44항 내지 제49항 중 어느 한 항에 있어서, 상기 라파마이신 또는 라파마이신 유사체가 대상에서 상당한 면역억제를 일으키지 않는 투여량으로 투여되는, 방법.

청구항 51

제39항 내지 제50항 중 어느 한 항에 있어서, 상기 종양 또는 암이 E2F 경로의 탈조절에 의해 특징화되는, 방법.

청구항 52

제39항 내지 제51항 중 어느 한 항에 있어서, 상기 종양 또는 암이 폐, 전립선, 결장직장, 유방, 갑상선, 신장 또는 간 종양 또는 암, 또는 백혈병인, 방법.

청구항 53

제41항 내지 제52항 중 어느 한 항에 있어서, 추가적인 치료제로 상기 대상을 치료하는 것을 추가로 포함하는, 방법.

청구항 54

제53항에 있어서, 상기 추가적인 치료제가 면역 조절제를 포함하는, 방법.

청구항 55

제53항에 있어서, 상기 추가적인 치료제가 사이클린-의존성 키나아제(CDK) 저해제를 포함하는, 방법.

청구항 56

재조합 아데노바이러스의 게놈이 E1A의 결실을 포함하고, EF1 α -루시퍼라제를 암호화하는, 재조합 아데노바이러스.

청구항 57

제56항에 있어서, 상기 게놈이 서열번호 27의 뉴클레오타이드 서열을 포함하는, 재조합 아데노바이러스.

청구항 58

제56항에 있어서, 상기 게놈이 간 특이적인 마이크로 RNA를 위한 하나 또는 그 이상의 결합 부위를 추가로 포함하는, 재조합 아데노바이러스.

청구항 59

제58항에 있어서, 상기 간 특이적인 마이크로 RNA를 위한 하나 또는 그 이상의 결합 부위가 E1A의 3'-UTR에 위치하는, 재조합 아데노바이러스.

청구항 60

제58항 또는 제59항에 있어서, 상기 간 특이적인 마이크로 RNA가 miR-122인, 재조합 아데노바이러스.

청구항 61

제58항 내지 제60항 중 어느 한 항에 있어서, 상기 게놈이 서열번호 28의 뉴클레오티드 서열을 포함하는, 재조합 아데노바이러스.

청구항 62

제56항 및 제58항 내지 제61항 중 어느 한 항에 있어서, 상기 게놈이 변형된 헥손(hexon) 단백질을 코딩하는, 재조합 아데노바이러스.

청구항 63

제62항에 있어서, 상기 변형된 헥손 단백질이 E451Q 치환을 포함하는, 재조합 아데노바이러스.

청구항 64

제62항 또는 제63항에 있어서, 상기 게놈이 서열번호 29의 뉴클레오티드 서열을 포함하는, 재조합 아데노바이러스.

청구항 65

서열번호 1 내지 31 및 68 내지 98 중 어느 하나의 뉴클레오티드 서열을 포함하는 재조합 헥산 분자.

청구항 66

제65항에 있어서, 상기 뉴클레오티드 서열이 서열번호 1 내지 31 및 68 내지 98 중 어느 하나의 서열로 이루어지는, 재조합 헥산 분자.

청구항 67

제65항 또는 제66항의 핵산 분자를 포함하는 벡터.

청구항 68

제67항의 벡터를 포함하는 형질전환세포.

청구항 69

제9항 내지 제16항 중 어느 한 항의 재조합 아데노바이러스 및 라파마이신 또는 이의 유사체를 포함하는 키트.

발명의 설명

기술 분야

[0001] 관련 출원에 대한 상호 참조

[0002] 본 출원은 그 전문이 본원에 참고로 인용된 2014년 9월 24일자로 출원된 미국 가출원 제62/054,724호의 우선권을 주장한다.

[0003] 분야

[0004] 본 발명은 종양세포에서 용균 복제를 선택적으로 겪는 E1A, E4orf1 및/또는 E4orf6/7의 하나 또는 그 이상의 변형을 포함하는 재조합 아데노바이러스에 관한 것이다. 상기 재조합 아데노바이러스는 종양세포에서 전신성 전달 및 흡수를 가능하게 하기 위해 섬유, 핵손 또는 캡시드 단백질에서 선택적으로 하나 또는 그 이상의 변형을 갖는다.

[0005] 연방 후원 연구 또는 개발에 관한 진술

[0006] 본 발명은 국립보건원(National Institutes of Health)이 수여한 5T32GM007240-35에 따른 정부지원으로 이루어졌다. 정부는 발명에 대해 특정 권리를 가지고 있다.

배경 기술

[0007] 암은 해마다 50만명 이상의 사망자를 발생시키는, 복잡하고 사람을 쇠약하게 하는 질병이다. 따라서, 보다 효과적이고, 선택적이며, 안전한 암 치료법이 절실히 필요하다. 화학요법 및 수술과 같은 보편적인 생명을 위협하는 질병에 대한 기존 치료법은 모든 악성세포를 거의 제거하지 못하며, 종종 치료효과를 상회할 수 있는 해로운 부작용을 나타낸다.

[0008] 현재 암 치료법의 단점을 상당 부분 해결할 수 있는 잠재력을 지닌 한 가지 접근법은 종양 살상 아데노바이러스 치료법이다(Pesonen *et al.*, *Molecular Pharmaceutics* 8(1):12-28, 2010). 아데노바이러스(Ad)는 자체적으로 복제할 수 있는 생물학적 머신이다. 아데노바이러스는 단백질 코트에 쌓인 선형 이중가닥 36kb DNA 게놈으로 구성된다. 아데노바이러스는 세포 복제용 시스템(machinery)을 침범하고 이용하여 번식하고, 조립시, 용균 세포살상을 유도하여 주변세포로 퍼진다. 이와 매우 유사한 세포 조절제는 암의 돌연변이를 표적으로 한다. 이는 유도된 미사일(missile), 특히 종양세포에서 감염 및 복제와 같은 역할을 하는 합성 바이러스를 만들 수 있으며, 가능한 저항을 극복하면서 원격전이를 찾고, 파괴할 수 있는 수천개의 바이러스 자손을 방출하도록 세포를 용해시킨다. 따라서, 종양 살상 바이러스의 설계목표는 암세포에서 특이적으로 복제하는 바이러스를 생성하는 것이지만, 정상세포는 손상시키지 않는다. 그러나 암세포에서 선택적으로 복제할 수 있는 바이러스를 설계하는 데는 상당한 어려움이 있었다. 따라서, 암세포에서 선택적으로 복제하는 추가적인 바이러스에 대한 필요성이 남아있다.

발명의 내용

[0009] 요약

[0010] E2F가 탈조절된 종양세포에서 선택적으로 복제하는 재조합 아데노바이러스가 개시된다. 상기 재조합 아데노바이

러스는 종양세포와 비교하여 정상세포에서 복제 결함을 나타내며, 변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 변형된 또는 결실된 E4orf6/7 단백질, 또는 이의 임의의 조합을 암호화하는 게놈을 갖는다. 상기 재조합 아데노바이러스의 게놈은 선택적으로 추가로 재조합 아데노바이러스를 특정 세포 유형으로 표적화, 간으로부터 바이러스를 비표적화(detarget), 간에서 바이러스 복제를 저해 및/또는 기존의 중화 항체를 회피하는 추가적인 변형을 암호화한다.

- [0011] 본 발명은 변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 변형된 또는 결실된 E4orf6/7 단백질, 또는 이들의 임의의 조합을 암호화하는 게놈을 가지며, 종양세포와 비교하여 정상세포에서 복제 결함을 갖는 재조합 아데노바이러스를 제공한다.
- [0012] 일부 실시예에서, 상기 재조합 아데노바이러스 게놈은 유도성 재표적화(retargeting)를 위한 변형을 추가로 암호화한다. 예를 들어, 상기 게놈은 선택적으로 FK506 결합 단백질(FKBP)에 융합된 표적 리간드, 및 야생형 FKBP-라파마이신 결합(FRB) 단백질 또는 T2098L 치환을 포함하는 돌연변이의 FRB 단백질에 융합된 아데노바이러스 섬유 단백질을 암호화한다.
- [0013] 일부 실시예에서, 상기 재조합 아데노바이러스 게놈은 아데노바이러스를 간으로부터 비표적화하는 변형을 추가로 암호화한다. 일부 예에서, 상기 게놈은 핵소 단백질의 변형을 암호화하고 및/또는 간 특이적인 마이크로 RNA를 위한 하나 또는 그 이상의 결합 부위를 포함한다.
- [0014] 일부 실시예에서, 상기 재조합 아데노바이러스 게놈은 바이러스를 다른 세포 수용체로 다시 보내는 키메라 섬유 단백질을 암호화한다.
- [0015] 일부 실시예에서, 상기 재조합 아데노바이러스 게놈은 기존의 중화 항체를 회피하기 위해 캡시드가 교환된 아데노바이러스를 암호화한다. 일부 예에서, 상기 게놈의 E1, E3 및 E4 영역은 제1 아데노바이러스 혈청형으로부터 유래되고, 상기 게놈의 E2B, L1, L2, L3, E2A 및 L4 영역은 제2 아데노바이러스 혈청형으로부터 유래된다.
- [0016] 추가로 재조합 리포터 아데노바이러스 유전자 발현 벡터가 제공되며, 여기서 상기 재조합 아데노바이러스의 게놈은 E1의 결실을 포함하고, EF1 α 프로모터가 있는 GFP-루시페라제 융합 단백질을 암호화한다. 일부 실시예에서, 상기 아데노바이러스 발현 벡터는 3'-UTR에서 간 특이적인 마이크로 RNA 결합 부위를 추가로 포함한다.
- [0017] 또한, 개시된 재조합 아데노바이러스를 포함하는 조성물이 본원발명에서 제공된다. 또한, 재조합 핵산 분자 및 재조합 아데노바이러스 게놈의 서열을 포함하는 벡터가 제공된다.
- [0018] 본 명세서에 개시된 재조합 아데노바이러스(또는 이의 조성물)를 투여함으로써 종양세포의 생존을 저해하고, 종양진행을 저해하며, 종양의 부피를 감소시키고, 암을 가진 개체를 치료하는 방법이 본 개시내용에 의해 추가로 제공된다.
- [0019] 본 명세서의 상기 및 다른 목적 및 특징은 첨부된 도면을 참조하여 진행되는 이하의 상세한 설명으로부터 더욱 명백해질 것이다.

도면의 간단한 설명

- [0020] 도 1A 및 1B는 재조합 아데노바이러스가 감염된 세포의 아데노바이러스 E1A의 발현을 나타내는 세포 용해물의 면역블롯을 나타낸 도이다. 세포는 AdSyn-CO102(야생형), AdSyn-CO181(E1A Δ LXCXE/ Δ E4orf6/7), AdSyn-CO189(E1A Δ LXCXE) 또는 ONYX-838(E1A Δ CR2)으로 감염되지 않거나(mock), 감염되었다. 도 1A에서, 휴지기상태의 인간 초대 소기도 상피세포(SAEC)는 MOI 10에서 감염되었고, 용해물은 E1A 발현에 대해 분석되었다. AdSyn-CO102에 감염된 세포에서, 감염 후기에 E1A 수준이 감소했다. 유사하게, AdSyn-CO189 또는 ONYX-838에 감염된 세포에서, 감염 후기에 E1A 수준이 감소했다; 그러나, 더 이른 시점에서, E1A의 발현은 AdSyn-CO102가 감염된 세포에서의 E1A의 발현보다 더 크다. 반면, AdSyn-CO181에 감염된 세포는 감염을 통해 더욱 강력하고 지속적인 E1A의 발현을 나타냈으며, 이는 아데노바이러스 생활사를 통해 진행하지 못함을 나타낸다. 도 1B에서, 융합성 폐 선암세포(A549)는 MOI 30에서 감염되었고, 용해물은 E1A 발현에 대해 분석되었다. 모든 감염된 세포에서 감염 후기에 E1A 수준이 감소되었고, 이는 전형적인 아데노바이러스 생활사가 진행되었음을 나타낸다.
- 도 2A 및 2B는 감염된 원발성 종양 및 종양세포에서 세포 사이클린(사이클린) 및 아데노바이러스 후기 단백질의 발현을 나타내는 세포 용해물의 면역블롯을 나타낸 도이다. 세포는 AdSyn-CO102(야생형), AdSyn-CO181(E1A Δ LXCXE/ Δ E4orf6/7), AdSyn-CO189(E1A Δ LXCXE), AdSyn-CO210(Δ E4orf6/7), 또는 ONYX-838(E1A Δ CR2)으로 감염되지 않거나(mock), 감염되었다. 도 2A에서, SAEC는 MOI 10에서 감염되었다. 야생형 바이러스 및 E1A 돌연변

이만 있는 바이러스와 달리, AdSyn-C0181은 E2F 의존성 세포주기 표적인 S기 사이클린, 사이클린 A 및 사이클린 B를 활성화시키지 못했다. 또한, AdSyn-E4orf6/7 돌연변이를 갖는 AdSyn-C0181 및 AdSyn-C0210은 후기 단백질 발현 및 복제에 결함이 있었다. AdSyn-C0210에서는 상기의 두 가지 결함이 모두 적게 나타났다. 도 2B에서, A549 세포는 MOI 30에서 감염되었다. 감염된 초대 세포와 달리, 후기 구조 단백질의 발현에는 뚜렷한 결함이 없었으며, 사이클린 A 및 사이클린 B는 모든 감염된 A549 샘플에 남아있었다.

도 3A 및 3B는 감염된 SAEC 및 A549 세포에서 각각 세포 DNA를 정량화하는 FACS 히스토그램을 나타낸 도이다. 세포는 AdSyn-C0102(야생형), AdSyn-C0181(E1A ΔLXCXE/ΔE4orf6/7), AdSyn-C0189(E1A ΔLXCXE) 또는 AdSyn-C0210(ΔE4orf6/7)으로 감염되지 않거나(mock), 감염되었으며, 감염 후 48시간에 수거되었다. 감염되지 않은 세포의 DNA 함량은 백그라운드 프로파일에 표시된다. Y축은 세포의 상대적인 수이며, X축은 세포의 프로피디움 요오드화물(PI)에서 나온 형광으로, 이는 DNA의 양에 비례한다. 도 3A에서, 휴지기상태의 SAEC는 MOI 10에서 감염되었다. AdSyn-C0181 감염은 감소된 바이러스 복제와 관련이 있는 AdSyn-C0102에 비해 SAEC에서 강한 DNA 복제 결함을 나타냈다. 보통의 결함은 상기 시점에서 AdSyn-C0210에 감염된 SAEC에서도 나타났다. 도 3B에서, A549 세포는 MOI 30에서 감염되었다. 이들 세포에서는 어떠한 돌연변이 바이러스 감염에서도 뚜렷한 DNA 복제결함이 나타나지 않았다.

도 4A 및 4B는 감염된 SAEC 및 A549 세포의 아데노바이러스 버스트(burst)를 각각 나타내는 그래프이다. 세포는 AdSyn-C0102(야생형), AdSyn-C0181(E1A ΔLXCXE/ΔE4orf6/7), AdSyn-C0189(E1A ΔLXCXE), AdSyn-C0210(ΔE4orf6/7) 또는 ONYX-838(E1A ΔCR2)으로 감염되지 않거나(mock), 감염되었으며, 감염 후 48시간 및 72시간에 배지가 수거되었다. 배지내의 감염성 바이러스 입자는 ELISA로 정량화되었다. 도 4A에서, 휴지기상태의 SAEC는 MOI 10에서 감염되었다. AdSyn-C0181 및 AdSyn-C0210 감염 모두 AdSyn-C0102에 비해 SAEC에서 강한 복제결함을 나타냈다. 도 4B에서, A549 세포는 MOI 30에서 감염되었다. AdSyn-C0210을 제외하고는, 이들 세포에서 바이러스의 복제결함이 나타나지 않았다.

도 5A 및 5B는 감염 7일 후, 감염된 SAEC 및 A549 세포의 세포 생존율을 각각 나타낸 그래프이다. 세포는 AdSyn-C0102(야생형), AdSyn-C0181(E1A ΔLXCXE/ΔE4orf6/7), AdSyn-C0189(E1A ΔLXCXE), AdSyn-C0210(ΔE4orf6/7) 또는 ONYX-838(E1A ΔCR2)가 연속희석법으로 감염되지 않거나(mock), 감염되었으며, 세포 대사 활성은 WST-1 분석으로 정량화되었다. 도 5A에서, AdSyn-C0102와 비교하여, AdSyn-C0181은 SAEC에서 세포 살상능력을 감소시켰다. 도 5B에서, 실험한 돌연변이 바이러스는 야생형 바이러스와 비교하여 세포살상에는 결함이 없었다.

도 6A는 세포 용해물의 면역블롯을 나타낸 도이다. MOI 10에서 감염된 정상적인 인간 정상세포(NHA)로 면역블롯을 수행하여 아데노바이러스 후기 구조 단백질 발현 및 세포 사이클린 유도를 검출하였다. AdSyn-C0181은 사이클린 A를 유도하지 않으며, 복제결함을 나타내는 AdSyn-C0102에 비해 감소 및 지연된 후기 바이러스 단백질을 발현한다.

도 6B 및 6C는 NHA에서 AdSyn-C0181이 약독화된 감염을 나타내는 그래프이다. 도 6B에서, NHA는 바이러스 패널(panel)에 의해 감염되었으며, 배지내 감염 입자의 수는 감염 후 48시간 및 72시간에 정량화되었다. AdSyn-C0181 및 AdSyn-C0189는 NHA에서 AdSyn-C0102에 비해 복제결함을 보였다. 도 6C에서, DNA 복제는 감염 48시간 후 MOI 10에서 감염된 NHA에서 PI FACS에 의해 정량화되었다. 감염되지 않은 세포의 DNA 함량은 히스토그램의 백그라운드 프로파일에 표시된다. Y축은 세포의 상대적인 수이며, X축은 핵산의 양에 비례하는 세포내 PI에서 나온 형광이다. AdSyn-C0181은 감소된 바이러스 복제와 관련이 있는 AdSyn-C0102와 비교하여 DNA 복제의 유도를 감소시켰다.

도 7A는 감염된 교모세포종 U87 세포로부터 얻은 세포 용해물의 면역블롯을 나타낸 도이다. MOI 20에서 감염된 U87 세포로 면역블롯을 수행하여 아데노바이러스 후기 구조 단백질 발현 및 세포 사이클린 유도를 검출하였다.

도 7B 및 7C는 교모세포종 U87 세포에서 복제결함이 없는 재조합 아데노바이러스를 나타내는 그래프이다. 도 7B에서, U87 세포는 바이러스 패널(panel)에 의해 감염되었으며, 배지내 감염 입자의 수는 감염 후 48시간 및 72시간에 정량화되었다. 도 7C에서, DNA 복제는 감염 48시간 후 MOI 20에서 감염된 U87 세포에서 PI FACS에 의해 정량화되었다. 감염되지 않은 세포의 DNA 함량은 히스토그램의 백그라운드 프로파일에 표시된다. Y축은 세포의 상대적인 수이며, X축은 핵산의 양에 비례하는 세포내 PI에서 나온 형광이다.

도 8A는 감염된 교모세포종 U118 세포로부터 얻은 세포 용해물의 면역블롯을 나타낸 도이다. MOI 20에서 감염된 U118 세포로 면역블롯을 수행하여 아데노바이러스 후기 구조 단백질 발현 및 세포 사이클린 유도를 검출하였다.

도 8B 및 8C는 교모세포종 U118 세포에서 복제결합이 없는 재조합 아데노바이러스를 나타내는 그래프이다. 도 8B에서, U118 세포는 바이러스 패널(panel)에 의해 감염되었으며, 배지내 감염 입자의 수는 감염 후 48시간 및 72시간에 정량화되었다. 도 8C에서, DNA 복제는 감염 48시간 후 MOI 20에서 감염된 U118 세포에서 PI FACS에 의해 정량화되었다. 감염되지 않은 세포의 DNA 함량은 히스토그램의 백그라운드 프로파일에 표시된다. Y축은 세포의 상대적인 수이며, X축은 핵산의 양에 비례하는 세포내 PI에서 나온 형광이다.

도 9A는 감염된 인간 섬유아세포(IMR90 세포)로부터 얻은 세포 용해물의 면역블롯을 나타낸 도이다. MOI 10에서 감염된 섬유아세포로 면역블롯을 수행하여 후기 구조 단백질 발현을 검출하였다. AdSyn-C0181은 복제결합을 나타내는 AdSyn-C0102에 비해 감소 및 지연된 후기 바이러스 단백질을 발현한다.

도 9B 및 9C는 AdSyn-C0181이 IMR90 세포에서 보통의 복제 결함을 갖는 것을 보여주는 그래프이다. 도 9B에서, 섬유아세포는 바이러스 패널(panel)에 의해 감염되었으며, 배지내 감염 입자의 수는 감염 후 48시간 및 72시간에 정량화되었다. 상기 시점에서 상기 분석법을 사용한 결과, 변형된 아데노바이러스의 복제 능력에는 명확한 차이가 없었다. 도 9C에서, DNA 복제는 감염 48시간 후 MOI 20에서 감염된 섬유아세포에서 PI FACS에 의해 정량화되었다. 감염되지 않은 세포의 DNA 함량은 히스토그램의 백그라운드 프로파일에 표시된다. Y축은 세포의 상대적인 수이며, X축은 핵산의 양에 비례하는 세포내 PI에서 나온 형광이다. AdSyn-C0181은 감소된 바이러스 복제와 관련이 있는 AdSyn-C0102와 비교하여 DNA 복제의 유도를 감소시켰다.

도 10은 감염된 1차 NHA 세포의 감염 10일 후 세포 생존능을 나타내는 그래프이다. 세포는 야생형 또는 돌연변이 바이러스가 연속희석법으로 감염되었다. 대사 활성은 WST-1 분석에 의해 정량화되었다. 세포 당 3 내지 10개의 감염성 입자에서 두 그룹의 바이러스가 뚜렷하게 나타났으며, 그래프에서 "더 적은 살상(less killing)"과 "더 많은 살상(more killing)"으로 분류되었다. 더 적은 세포 살상을 유도한 바이러스는 Rb 결합 돌연변이 및 E4orf6/7의 결실이 있는 E1A를 가진다. 더 많은 살상을 유도한 바이러스는 야생형 E1 또는 야생형 E4를 가진다.

도 11은 감염된 휴지기상태 SAEC-hTERT 세포의 감염 9일 후 세포 생존능을 나타내는 그래프이다. 세포는 야생형 또는 돌연변이 바이러스가 연속희석법으로 감염되었다. 대사 활성은 WST-1 분석에 의해 정량화되었다. 세포 당 3 내지 10개의 감염성 입자에서 두 그룹의 바이러스가 뚜렷하게 나타났으며, 그래프에서 "더 적은 살상(less killing)"과 "더 많은 살상(more killing)"으로 분류되었다. 더 적은 세포 살상을 유도한 바이러스는 Rb 결합 돌연변이 및 E4orf6/7의 결실이 있는 E1A를 가진다. 더 많은 살상을 유도한 바이러스는 야생형 E1 또는 야생형 E4를 가진다.

도 12는 감염된 증식하는 SAEC-hTERT 세포의 감염 9일 후 세포 생존능을 나타내는 그래프이다. 세포는 야생형 또는 돌연변이 바이러스가 연속희석법으로 감염되었다. 대사 활성은 WST-1 분석에 의해 정량화되었다. 세포 당 3 내지 10개의 감염성 입자에서 두 그룹의 바이러스가 뚜렷하게 나타났으며, 그래프에서 "더 적은 살상(less killing)"과 "더 많은 살상(more killing)"으로 분류되었다. 더 적은 세포 살상을 유도한 바이러스는 Rb 결합 돌연변이 및 E4orf6/7의 결실이 있는 E1A를 가진다. 더 많은 살상을 유도한 바이러스는 야생형 E1 또는 야생형 E4를 가진다.

도 13은 감염된 A549 세포의 감염 7일 후 세포 생존능을 나타내는 그래프이다. 세포는 야생형 또는 돌연변이 바이러스가 연속희석법으로 감염되었다. 대사 활성은 WST-1 분석에 의해 정량화되었다.

도 14는 감염된 사람 유방암세포(MDA MB 231)의 감염 7일 후 세포 생존능을 나타내는 그래프이다. 세포는 야생형 또는 돌연변이 바이러스가 연속희석법으로 감염되었다. 대사 활성은 WST-1 분석에 의해 정량화되었다.

도 15는 감염된 교모세포종 세포(U87)의 감염 7일 후 세포 생존능을 나타내는 그래프이다. 세포는 야생형 또는 돌연변이 바이러스가 연속희석법으로 감염되었다. 대사 활성은 WST-1 분석에 의해 정량화되었다.

도 16은 도 10 내지 15에 나타난 감염된 1차 NHA, SAEC-hTERT(휴지기상태), SAEC-hTERT(증식하는), A549, MDA MB 231 및 U87 세포에 대한 세포 생존능을 정량화한 표이다.

도 17은 감염된 U2OS 세포(기능적인 p53 및 Rb를 가짐) 및 SaOS2 세포(비기능적인 p53 및 Rb를 가짐)에 대한 세포 생존능을 정량화한 표이다. 세포는 야생형 또는 돌연변이 바이러스가 연속희석법으로 감염되었다. 대사 활성은 WST-1 분석에 의해 정량화되었다.

도 18A는 FRB 도메인과 라파마이신 및 FKBP12와의 결합 경계면을 나타내는 도이다. 돌연변이된 FRB 도메인(FRB^{*}; mTOR 잔기 T2098L)은 라파마이신 또는 라파마이신 동족체(homolog) AP21967과 FRB/FKBP12 헤테로다имер를 형성하도록 유도된다. 도 18B는 FRB-섬유(AdSyn-C0205; 서열번호 88)를 갖는 EGFR-표적화 바이러스가 라파마

이신의 존재하에서 MDA MB 453 유방암세포의 향상된 전달을 가졌지만 AP21967은 그렇지 않음을 보여주는 그래프이다. FRB^{*}-섬유(AdSyn-CO220; 서열번호 98)를 갖는 EGFR-표적화 바이러스는 라파마이신 또는 AP21967의 존재하에서 MDA MB453 세포의 형질도입이 향상되었다. 도 18C는 표적인 p70 S6K의 인산화를 차단하는 mTOR 저해제 라파마이신의 활성을 나타내는 면역블롯이다. 라파마이신 동족체 AP21967은 표적화 농도에서 mTOR를 저해하지 않는다. 도 18D는 감염된 전이성 유방암세포 HS578T의 감염 9일 후 세포 생존능을 나타내는 그래프이다. 세포는 AdSyn-CO312가 연속희석법으로 감염되었다. 감염 후 24, 48, 72 및 96시간에 50nM의 신선한 라파마이신 또는 AP21967이 첨가되었다. 대사 활성은 WST-1 분석에 의해 정량화되었고, 일치하는 약물 치료법으로 감염되지 않은 세포에 대해 정상화되었다. AdSyn-CO312는 E1A ΔLXCXE, ΔE4orf6/7, ΔE3-RID α/β, ΔE3-14.7k의 종양 살상 돌연변이를 가지며, 라파마이신-또는 AP21967-의존성 EGFR-표적화 유전자 EGFRVHH-FKBP 및 FRB^{*}-섬유를 발현한다. 라파마이신 또는 AP21967이 처리된 EGFR-표적화된 종양 살상 바이러스에 감염된 세포는 표적화되지 않은 경우에 비해 살상이 강화되었다. 도 18E는 감염된 전이성 유방암세포 HS578T의 감염 9일 후 세포 생존능을 나타내는 그래프이다. 세포는 연속적으로 희석된 AdSyn-CO313d1로 감염되었다. 감염 후, 24, 48, 72 및 96시간에 50nM의 신선한 라파마이신 또는 AP21967이 첨가되었다. 대사 활성은 WST-1 분석에 의해 정량화되었고, 일치하는 약물 치료법으로 감염되지 않은 세포에 대해 정상화되었다. AdSyn-CO313은 E1A ΔLXCXE, ΔE4orf6/7, ΔE3-RID α/β, ΔE3-14.7k의 종양 살상 돌연변이를 가지며, 라파마이신-의존성 EGFR-표적화 유전자 EGFRVHH-FKBP 및 FRB-섬유를 발현한다. 라파마이신이 처리된 EGFR-표적화 종양 살상 바이러스에 감염된 세포는 표적화되지 않은 경우에 비해 살상이 강화되었다.

도 19는 HS578T를 피하 이종 이식한 마우스에서 평균적인 종양의 부피를 나타내는 그래프이다. 종양이 생긴 마우스의 종양내로 AdSyn-CO313가 주사된 후, 주기적으로 복강내에 8 mg/kg의 라파마이신(n = 5) 또는 비히클(vehicle) 대조군(n = 4)이 주사되거나 비히클 대조군이 종양내로 주사된 후, 주기적으로 복강내에 8 mg/kg의 라파마이신(n = 5) 또는 비히클 대조군(n = 4)이 주사되었다. 감염은 초기 감염 후 19일부터 4일 간격으로 같은 그룹에서 3번 이상 반복하여 감염되었다. AdSyn-CO313은 E1A ΔLXCXE, ΔE4orf6/7, ΔE3-RID α/β 및 ΔE3-14.7k의 종양 살상 돌연변이를 가지며, 라파마이신-의존성 EGFR-표적화 유전자 EGFRVHH-FKBP 및 FRB-섬유를 발현한다. EGFR-표적화 종양 살상 바이러스를 라파마이신과 함께 투여한 마우스에서 종양은 최상의 반응을 나타냈다.

도 20은 감염된 전이성 유방암세포 HS578T의 감염 9일 후 세포 생존능을 나타내는 그래프이다. 세포는 연속적으로 희석된 AdSyn-CO335로 감염되었다. 감염 후, 24, 48, 72 및 96시간에 50nM의 신선한 라파마이신 또는 AP21967이 첨가되었다. 대사 활성은 WST-1 분석에 의해 정량화되었고, 일치하는 약물 치료법으로 감염되지 않은 세포에 대해 정상화되었다. AdSyn-CO335는 E1A ΔLXCXE, ΔE4orf6/7, ΔE3-RID α/β 및 ΔE3-14.7k의 종양 살상 돌연변이를 가지며; 간을 표적화하지 않는 E451Q 핵손 변형을 가지며; 라파마이신- 또는 AP21967-의존성 EGFR-표적화 유전자 EGFRVHH-FKBP 및 FRB^{*}-섬유를 발현한다. 라파마이신 또는 AP21967이 처리된 EGFR-표적화 종양 살상 바이러스에 감염된 세포는 표적화되지 않은 경우에 비해 살상이 강화되었다.

도 21A 및 21B는 HS578T를 피하 이종 이식한 마우스에서 평균적인 종양의 부피를 나타내는 그래프이다. 도 21C 및 21D는 HS578T를 피하 이종 이식한 마우스에서 종양 부피의 변화를 배수 값으로 나타낸 그래프이다. 종양이 생긴 마우스의 종양내로 Syn-CO335가 4일마다 세 차례씩 주사된 후, 다음날부터 격일로 복강내에 2 또는 8 mg/kg의 라파마이신(각각 n = 8 및 n = 8), 또는 비히클(vehicle) 대조군(n = 6)이 주사되거나; 종양이 생긴 마우스의 종양내로 AdSyn-CO442가 4일마다 세 차례씩 주사된 후, 다음날부터 격일로 복강내에 2 또는 8 mg/kg의 라파마이신(각각 n = 6 및 n = 6)이 주사되거나; 종양이 생긴 마우스의 종양내로 비히클 대조군이 4일마다 세 차례씩 주사된 후, 다음날부터 격일로 복강내에 2 또는 8 mg/kg의 라파마이신(각각 n = 6 및 n = 6)이 주사되었다. EGFR-표적화 종양 살상 바이러스를 8 mg/kg의 라파마이신과 함께 투여한 마우스에서 종양은 최상의 반응을 나타냈다.

도 22A 내지 22C는 간에서 아데노바이러스가 매개된 유전자 발현의 제거 및 간으로의 아데노바이러스 감염의 비표적화(detargeting)를 나타내는 사진이다. 도 22A에 나타난 바와 같이, E1 영역이 결여된 아데노바이러스 벡터인 AdSyn-CO199는 전신성 감염 후 간에서 독점적으로 루시페라제를 높은 수준으로 발현한다. 도 22B에 나타난 바와 같이, 간 특이적인 microRNA miR122가 루시페라제 발현을 제거한다는 것을 제외하고는 AdSyn-CO199와 일치하는 AdSyn-CO200가 마우스 전신에 감염되면 루시페라제 신호가 사라진다. 도 22C에 나타난 바와 같이, 간으로부터 비표적화하는 아데노바이러스 핵손 단백질이 돌연변이 E451Q를 가지는 것을 제외하고는 AdSyn-CO200과 일치하는 AdSyn-CO171이 마우스 전신에 감염되면 루시페라제의 발현은 비장에서 검출될 수 있다.

도 23A 내지 23E는 섬유 단백질 구조의 모식도 및 다양한 세포 유형을 더 잘 변환하는 키메라 섬유 단백질로 바이러스를 생성하기 위한 두 가지 아데노바이러스 혈청형으로부터의 모듈을 조합하여 생성된 재조합 아데노바이러스를 특징으로 하는 일련의 그래프 및 이미지를 나타내는 도이다.

도 24A는 캡시드가 교환된 키메라 바이러스를 생성하기 위한 모듈변경을 나타내는 도이다. 도 24B는 특정 돌연변이 바이러스 및 바이러스의 E1, 코어(core), E3 및 E4 모듈 변경사항을 나열한 표이다.

도 25A 내지 25E는 캡시드가 교환된 바이러스로부터 단백질 함량 및 발현을 나타내는 일련의 면역블롯을 나타낸 도이다.

도 26A 내지 26C는 캡시드가 교환된 바이러스가 항-Ad5 항체를 함유하는 인간 혈청에 의해 중화되지 않음을 보여주는 일련의 그래프이다.

도 27은 종 A(Ad12; 서열번호 47), 종 B(Ad7; 서열번호 48), 종 C(Ad2 및 Ad5; 각각 서열번호 49 및 서열번호 50), 종 D(Ad9; 서열번호 51), 종 E(Ad4; 서열번호 52), 종 F(Ad40; 서열번호 53) 및 종 G(Ad52; 서열번호 54)를 포함하는 인간 아데노바이러스 종으로부터의 E1A 서열을 정렬한 도이다. 본 명세서에 개시된 종양 살상 아데노바이러스 조성물에서 발견되는 돌연변이 부위인 Y47이 개략적으로 설명된다. 회색 상자로 강조 표시된 것은 강력한 E1A-Rb 상호작용에 필요한 C124가 포함된 LXCXE 모티프이다.

도 28은 종 A(Ad12; 서열번호 55), 종 B(Ad7; 서열번호 56), 종 C(Ad2 및 Ad5; 각각 서열번호 57 및 서열번호 58), 종 D(Ad9; 서열번호 59), 종 E(Ad4; 서열번호 60) 및 종 G(Ad52; 서열번호 61)를 포함하는 인간 아데노바이러스 종으로부터의 E4orf1 서열을 정렬한 도이다. 회색 상자로 강조 표시된 것은 E4orf1이 PI3K를 상향 조절하고 세포 증식의 음성 조절을 방지하는 PDZ 결합 모티프이다.

도 29는 라파마이신-의존성 EGFR-표적화 성분을 갖는 합성 아데노바이러스로 감염된 293-E4 세포로부터의 단백질 발현의 면역블롯을 나타내는 도이다. 293-E4 세포는 Adassembly 야생형 Ad(AdSyn-C0170), 표적화-변형된 AdSyn-C0205, 또는 대조군 바이러스인 AdSyn-C0206 또는 AdSyn-C0207에 의해 MOI 10으로 감염되었다. 라파마이신-조절된 EGFR-표적화 바이러스 AdSyn-C0205를 위해, GFP 리포터는 감염의 표지자로서 E1A에 융합되었다. E3B 전사체는 RID α , RID β , 및 14.7k를 제거함으로써 변형되었으며, EGFRVHH-FKBP를 발현하는데 사용되었다. AdSyn-C0206은 AdSyn-C0205의 모든 변형을 포함하지만, 야생형 섬유를 유지하므로 FRB 도메인을 갖지 않는다. AdSyn-C0207은 AdSyn-C0206의 모든 변형을 가지고 있지만, 야생형 E3 영역을 유지하므로 FKBP-융합 유전자를 갖지 않는다. 섬유의 이동에서 변화는 AdSyn-C0207 및 AdSyn-C0205에서 90개의 아미노산 FRB 도메인의 삽입을 보여준다. EGFRVHH-FKBP 융합 단백질의 발현은 FKBP12에 대해 조사함으로써 검출되었다. 후기 단백질 발현의 시간 경과에 따라, 캡시드-돌연변이, 또는 GFP-E1A 리포터를 가진 EGFRVHH-FKBP 발현 바이러스의 복제에 있어서 명백한 결함이 Adassembly 야생형 Ad와 비교하여 나타나지 않았다.

도 30은 비히클(비감염), 대조군 바이러스(AdSyn-C0442), 또는 종양 살상 아데노바이러스(AdSyn-C0335)의 종양 내 3회 주사 및 3주간 격일로 라파마이신(0, 2 또는 8 mg/kg)의 종양내 공투여 후, 28일째의 HS578T 이종 이식편 종양반응을 나타낸 워터폴(waterfall) 플롯을 나타낸 도이다. 왼쪽에서 오른쪽으로 (a) 감염되지 않았고, 라파마이신이 투여되지 않음; (b) 감염되지 않았고, 2 mg/kg의 라파마이신이 투여됨; (c) 감염되지 않았고, 8 mg/kg의 라파마이신이 투여됨; (d) AdSyn-C0335가 감염되었고, 라파마이신이 투여되지 않음; (e) AdSyn-C0335가 감염되었고, 2 mg/kg의 라파마이신이 투여됨; (f) AdSyn-C0335가 감염되었고, 8 mg/kg의 라파마이신이 투여됨; (g) AdSyn-C0442가 감염되었고, 2 mg/kg의 라파마이신이 투여됨; 및 (h) AdSyn-C0442가 감염되었고, 8 mg/kg의 라파마이신이 투여됨. N = 각 그룹에서 종양의 수. 각각의 열은 하나의 개별 종양을 나타내며, 치료 전 종양 부피와 관련하여 데이터가 표시된다.

도 31은 HS578T 이종 이식 조직의 면역조직화학염색(IHC)을 나타내는 도이다. HS578T를 피하 이종 이식한 마우스는 복강내에 100 μ l의 비히클, 2 mg/kg의 라파마이신 또는 8 mg/kg의 라파마이신이 주사됨으로써 치료되었다. 주사하고 1시간 후, 이종 이식 조직을 수득하여 10% 포르말린에 고정시키고, P70S6K의 포스포-T378 형태를 인식하는 항체로 IHC 염색을 위해 절편화했다. 트레오닌 389(P70S6K p-T389)에서 인산화된 P70S6K에 대해 염색된 HS578T 이종 이식편 조직 절편의 마이크로픽토그래프가 나타나있다. T389에서 P70S6K의 인산화는 mTOR 활성화의 특징이다. 치료가 되지 않은 조직 또는 낮은 아데노바이러스 EGFR-표적화 유효량(2 mg/kg)에서, mTOR 활성화는 P70S6K p-T389의 염색에 의해 입증된 바와 같이 저해되지 않는다. 보다 높은 아데노바이러스 EGFR-표적화 유효량(8 mg/kg)에서, mTOR 활성화는 저해되고, P70S6K p-T389 염색의 소실로 표시된다.

서열목록

수반되는 서열목록에 열거된 핵산 및 아미노산 서열은 뉴클레오티드 염기에 대한 표준 문자 약어 및 37 C.F.R. 1.822에 정의된 아미노산에 대한 3문자 코드를 사용하여 나타냈다. 핵산 서열 각각의 한 가닥만을 나타내지만, 상보적인 가닥은 표시된 가닥에 대한 임의의 언급에 의해 포함되는 것으로 이해된다. 서열목록은 2015년 9월 23일, 2.91 MB에 작성된 ASCII 텍스트 파일로 제출되며, 상기 파일은 여기에 참조로 포함된다. 수반되는 서열목록은 하기와 같다:

서열번호 1 내지 31 및 68 내지 98은 재조합 아데노바이러스의 뉴클레오티드 서열이다(표 1 내지 5; 실시예 6; 및 실시예 7 참조).

서열번호 32는 Ad5 E1A의 아미노산 서열이다.

서열번호 33은 Ad5 E1A ΔLXCXE의 아미노산 서열이다.

서열번호 34는 Ad5 E1A C124G의 아미노산 서열이다.

서열번호 35는 Ad5 E1A Δ2-11의 아미노산 서열이다.

서열번호 36은 Ad5 E1A Y47H C124G의 아미노산 서열이다.

서열번호 37은 Ad5 E1A Δ2-11 Y47H C124G의 아미노산 서열이다.

서열번호 38은 Ad5 E4orf1의 아미노산 서열이다.

서열번호 39는 Ad5 E4orf1ΔPDZb의 아미노산 서열이다.

서열번호 40은 Ad5 E4orf6/7의 아미노산 서열이다.

서열번호 41은 Ad5 섬유의 아미노산 서열이다.

서열번호 42는 Ad5 FRB-섬유의 아미노산 서열이다.

서열번호 43은 Ad5 FRB^{*}-섬유의 아미노산 서열이다.

서열번호 44는 EGFRVHH-GS-FKBP의 아미노산 서열이다.

서열번호 45는 Ad5 핵손의 아미노산 서열이다.

서열번호 46은 Ad5 핵손 E451Q의 아미노산 서열이다.

서열번호 47은 종 A (Ad12) E1A의 아미노산 서열이다.

서열번호 48은 종 B (Ad7) E1A의 아미노산 서열이다.

서열번호 49는 종 C (Ad2) E1A의 아미노산 서열이다.

서열번호 50은 종 C (Ad5) E1A의 아미노산 서열이다.

서열번호 51은 종 D (Ad9) E1A의 아미노산 서열이다.

서열번호 52는 종 E (Ad4) E1A의 아미노산 서열이다.

서열번호 53은 종 F (Ad40) E1A의 아미노산 서열이다.

서열번호 54는 종 G (Ad52) E1A의 아미노산 서열이다.

서열번호 55는 종 A (Ad12) E4orf1의 아미노산 서열이다.

서열번호 56은 종 B (Ad7) E4orf1의 아미노산 서열이다.

서열번호 57은 종 C (Ad2) E4orf1의 아미노산 서열이다.

서열번호 58은 종 C (Ad5) E4orf1의 아미노산 서열이다.

서열번호 59는 종 D (Ad9) E4orf1의 아미노산 서열이다.

서열번호 60은 종 E (Ad4) E4orf1의 아미노산 서열이다.

서열번호 61은 종 G (Ad52) E4orf1의 아미노산 서열이다.

서열번호 62는 Ad2의 뉴클레오티드 서열이다.

서열번호 63은 Ad5의 뉴클레오티드 서열이다.

서열번호 64는 Ad2 E4orf6/7의 아미노산 서열이다.

서열번호 65는 Ad5 E3-RID α 의 아미노산 서열이다.

서열번호 66은 Ad5 E3-RID β 의 아미노산 서열이다.

서열번호 67은 Ad5 E3-14.7K의 아미노산 서열이다.

발명을 실시하기 위한 구체적인 내용

I. 약어

Ad 아데노바이러스

CAR 콕사키(coxsackie) 아데노바이러스 수용체

CPE 세포변성 효과

EGFR 표피성장인자수용체

ELISA 효소면역측정법

FACS 형광표지세포분류

FKBP FK 506 결합 단백질

FRB FKBP-라파마이신 결합

hTERT 인간 텔로머라아제 역전사 효소

HuVEC 인간 혈관 내피세포

MAV-1 마우스 아데노바이러스 1

miR 마이크로 RNA

MOI 감염다중도

mTOR 라파마이신의 포유류 표적

NHA 정상적인 인간 성상세포

PET 양전자방출단층촬영

PI 프로피디움 요오드화물

PRP PET 리포터 프로브

Rb 망막아세포종(retinoblastoma)

SAEC 소기도 상피세포

WT 야생형

II. 용어 및 방법

달리 명시하지 않는 한, 기술적인 용어는 통상적인 사용법에 따라 사용된다. 분자 생물학에서 일반적인 용어의 정의는 Benja분 Lewin, *Genes V*, published by Oxford University Press, 1994(ISBN 0-19-854287-9); Kendrew *et al.*(eds.), *Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994(ISBN 0-632-02182-9); 및 Robert A. Meyers(ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995(ISBN 1-56081-569-8)에 기재되어있다.

본 명세서에 개시된 다양한 실시예들의 검토를 용이하게 하기 위해, 특정 용어에 대한 하기의 설명이 제공된다 :

- [0046] **아데노바이러스:** 라이너(liner), 이중가닥 DNA 게놈 및 정이십면체 캡시드를 갖는 비 외피성(non-enveloped) 바이러스이다. 현재 7종(종 A, B, C, D, E, F 및 G)으로 분류된 68종의 인간 아데노바이러스 혈청형이 있다. 아데노바이러스의 다른 혈청형은 호흡기 질환(주로 종 B 및 C), 결막염(종 B 및 D) 및/또는 위장염(종 F 및 G)을 유발하는 여러 유형의 질병과 관련된다.
- [0047] **투여:** 임의의 효과적인 경로에 의해 치료제(예를 들어, 재조합 바이러스)와 같은 제제를 개체에게 제공하기 위함이다. 예시적인 투여 경로는 주사(피하, 근육내, 피내, 복강내 및 정맥내), 경구, 관내, 설하, 직장, 경피, 비강내, 질 및 흡입경로를 포함하지만 이에 한정되지 않는다.
- [0048] **항체:** 항원의 에피토프(epitope)를 인식하고 결합(예를 들어, 특이적으로 인식하고 특이적으로 결합)하는 경쇄 및/또는 중쇄 면역글로불린 가변영역을 적어도 포함하는 폴리펩티드 리간드이다. 면역글로불린 분자는 중쇄 및 경쇄로 구성되며, 각각은 중쇄 가변영역(V_H) 및 경쇄 가변영역(V_L)으로 불리는 가변영역을 갖는다. V_H 영역 및 V_L 영역은 함께 항체에 의해 인식되는 항원을 결합시키는 역할을 한다.
- [0049] 항체는 손상되지 않은 면역글로불린 및 단일 도메인 항체(예를 들어, V_H 도메인 항체 또는 V_{HH} 항체), Fab 단편, Fab' 단편, $F(ab)'\_2$ 단편, 단일 가닥의 Fv 단백질("scFv"), 및 이황화물 안정화된 Fv 단백질("dsFv")와 같은 당업계에서 잘 알려진 항체의 변이체 및 부분(단편)을 포함한다. scFv 단백질은 면역글로불린의 경쇄 가변영역과 면역글로불린의 중쇄 가변영역이 링커에 의해 결합된 융합 단백질이며, dsFv에서는 가닥이 돌연변이되어 이황화 결합을 도입하여 가닥의 결합을 안정화시킨다. 용어 "항체"는 또한 키메라 항체(예를 들어, 인간화된 쥐 항체) 및 이종접합 항체(예를 들어, 이중 특이성 항체)와 같은 유전적으로 조작된 형태를 포함한다. 또한, *Pierce Catalog and Handbook*, 1994-1995(Pierce Chemical Co., Rockford, IL); Kuby, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997을 참조한다.
- [0050] **화학치료제:** 비정상적인 세포 성장에 의해 특징화되는 질병의 치료에서 치료적 유용성을 가지는 화학작용제이다. 상기 질병은 종양, 신생물 및 암뿐만 아니라 건선과 같은 과증식 성장에 의해 특징화되는 질병을 포함한다. 일 실시예에서, 화학치료제는 방사성 화합물이다. 당업계에서 기술자는 사용하는 화학치료제(예를 들어, Slapak and Kufe, *Principles of Cancer Therapy*, Chapter 86 in Harrison's Principles of Internal Medicine, 14th edition; Perry *et al.*, *Chemotherapy*, Ch. 17 in Abeloff, *Clinical Oncology* 2nd ed., © 2000 Churchill Livingstone, Inc; Baltzer, L., Berkery, R.(eds.): *Oncology Pocket Guide to Chemotherapy*, 2nd ed. St. Louis, Mosby-Year Book, 1995; Fischer, D.S., Knopf, M.F., Durivage, H.J.(eds): *Cancer Chemotherapy Handbook*, 4th ed. St. Louis, Mosby-Year Book, 1993 참조)를 쉽게 확인할 수 있다. 병용화학요법은 암 치료를 위한 하나 이상의 제제를 투여하는 것이다.
- [0051] **키메라:** 적어도 기원이 다른 두 부분으로 구성된다. 본 명세서에 개시된 "키메라 아데노바이러스"는 적어도 두 개의 상이한 혈청형(예를 들어, Ad5 및 아데노바이러스의 제2 혈청형)으로부터 유래된 유전물질 및/또는 단백질을 갖는 아데노바이러스이다. 이러한 맥락에서, "캡시드 교환된" 아데노바이러스는 캡시드 단백질이 하나의 혈청형 아데노바이러스로부터 유래되고 나머지 단백질이 다른 아데노바이러스 혈청형으로부터 유래된 키메라 아데노바이러스를 의미한다. 유사하게, "키메라 섬유"는 아데노바이러스의 적어도 두 개의 상이한 혈청형으로부터 유래된 아미노산 서열을 갖는 섬유 단백질이다. 예를 들어, 키메라 섬유는 Ad5로부터의 섬유 샤프트 및 아데노바이러스의 제2 혈청형으로부터의 섬유 노브로 구성될 수 있다(도 23A 참조).
- [0052] **접촉시키는:** 직접적인 물리적 연관에서의 배치이다; 고체 및 액체 형태 모두를 포함한다.
- [0053] **퇴행 변이체:** 본 명세서에 개시된 "퇴행 변이체"는 유전암호의 결과로서 퇴행된 서열을 포함하는 펩티드를 암호화하는 폴리뉴클레오티드를 의미한다. 20개의 천연 아미노산이 있으며, 대부분은 하나 이상의 코돈에 의해 특정된다. 따라서, 뉴클레오티드 서열에 의해 암호화되는 펩티드의 아미노산 서열이 변하지 않는 한, 펩티드를 암호화하는 모든 퇴행 뉴클레오티드 서열이 포함된다.
- [0054] **결실된:** "결실된" E4orf1 또는 E4orf6/7 단백질을 암호화하는 아데노바이러스 게놈은 E4orf1 또는 E4orf6/7 코딩서열의 완전한 결실, 또는 E4orf1 또는 E4orf6/7 단백질의 부재로 인한 부분적인 결실을 갖는 아데노바이러스를 의미한다.
- [0055] **E2F의 비조절:** 거의 모든 유형의 인간 암에서 발생하는 E2F 전사인자 및 하류 표적 유전자의 활성 증가를 의미한다. E2F 경로 활성 및 전사의 비조절은 Rb, p107 및 p130 종양 억제인자의 기능 상실 돌연변이 및 결실과 같

은 경로의 임의의 상류 구성요소에서 다양한 다른 돌연변이로 인해 발생할 수 있다. Rb는 확인된 최초의 종양 억제인자였고, 적어도 인간 종양의 1/3에서 결함되거나 돌연변이가 발생했다. 또한, p16 돌연변이 및/또는 후생학적 사일런싱(silencing)은 종양세포에서 E2F를 활성화시킬 수 있다. 사이클린 D 및 CDK4 돌연변이, 유전자 증폭 또는 과발현은 또한 인간 종양에서 조절되지 않은 E2F 활성을 초래할 수 있다. 또한, E2F는 EGFR, RTK, RAS, RAF, PI-3K, PTEN, RAF, MYC 등을 포함하는 성장인자 수용체 경로 돌연변이에 의해 활성화된다. p16^{INK4a}-사이클린 D:cdk4/6-RB-E2F 경로에서 돌연변이는 일반적으로 상호 배타적인 방식으로 발생하기 때문에 하나의 '히트(hit)'(예를 들어, p16)는 다른 것들에 의해 동반되지 않는다(예를 들어, Rb 돌연변이 또는 사이클린 D:cdk 과발현). 그러나 현재 대부분의 화학요법은 E2F 전사 표적을 저해하는 증식성 독극물이지만, 정상세포에도 독성을 지니고 있으며 종종 파괴적인 의인성 합병증을 일으킨다. 본 명세서에 개시된 바와 같이, 대안적인 치료 방법은 조절되지 않은 p16-사이클린 D:cdk4-RB-E2F 경로를 가진 암세포 병변에서 선택적인 용균 복제를 수행하는 바이러스를 사용하는 것이다.

[0056] E1A: 아데노바이러스 초기 영역 1A(E1A) 유전자 및 유전자로부터 발현된 폴리펩티드이다. E1A 단백질은 세포를 세포주기로 유도하여 바이러스 게놈 복제에 중요한 역할을 한다. 본 명세서에서 개시된 용어 "E1A 단백질"은 E1A 유전자로부터 발현된 단백질을 의미하며 용어는 임의의 아데노바이러스 혈청형에 의해 생성된 E1A 단백질을 포함한다. 일례로, 야생형 Ad5 E1A 단백질의 아미노산 서열은 서열번호 32로 기재되며, 변형된 Ad5 E1A 서열은 서열번호 33 내지 37로 본 명세서에서 제공된다. 또한, 다양한 다른 아데노바이러스 혈청형으로부터의 야생형 E1A 단백질 서열이 본 명세서에서 서열번호 47 내지 54로 기재된다. 일부 실시예에서, 변형된 E1A 단백질은 서열번호 32 내지 37 또는 서열번호 47 내지 54 중 어느 하나와 적어도 80%, 적어도 85%, 적어도 90%, 적어도 95%, 적어도 96%, 적어도 97%, 적어도 98%, 또는 적어도 99%의 서열 동일성을 갖는다. 본 명세서에서 고려된 변형된 E1A 단백질은 종양세포와 비교하여 정상세포에서 재조합 아데노바이러스의 복제 결함에 기여하는 단백질이다. 본 명세서에 개시된 변형된 E1A 단백질은 Ad5 E1A 단백질이다. 그러나, 상응하는 변형이 임의의 바람직한 혈청형에서 이루어질 수 있으며, 따라서 본 명세서에 포함된다. 예를 들어, 모든 종류의 인간 아데노바이러스는 Ad5에서 LTCHE(서열번호 32의 122 내지 126 잔기)에 상응하는 LXCXE 모티프를 포함한다. 유사하게, 2 내지 11 잔기 및 Y47H 및 C124G 치환의 결실은 Ad5와 관련하여 번호가 매겨지지만, 임의의 다른 혈청형으로 도입될 수 있다(모든 인간 아데노바이러스 종으로부터 E1A의 정렬을 제공하는 도 27 참조).

[0057] E3-RID α/RID β 및 E3-14.7k: E3 유전자로부터 조기 발현된 단백질이다. E3-RID α, E3-RID β, 및 E3-14.7k 단백질은 핵막에 국한되어 CD95(FasL 수용체)를 포함하는 다양한 수용체의 엔도시토시스(endocytosis) 및 분해를 일으키는 수용체 내재화(internalization) 및 분해 복합체(RID), 및 TNFR1 및 2(TNF/TRAIL 수용체)로 감염된 세포를 숙주 항바이러스 반응으로부터 보호한다. E3-RID α, E3-RID β, 및 E3-14.7k 코딩서열은 상기 순서대로 서로 인접한다. 본 명세서의 일부 실시예에서, E3-14.7k의 정지코돈에 대한 E3-RID α의 개시코돈은 결실되고, FKBP-융합 단백질의 코딩서열(예를 들어, AdSyn-CO312, AdSyn-CO313, AdSyn-CO335 및 AdSyn-CO440)로 대체되었다. Ad5 E3-RID α, E3-RID β, 및 E3-14.7k의 아미노산 서열은 본 명세서에서 서열번호 65 내지 67로 기재된다.

[0058] E4orf1: E4 유전자로부터 생산된 아데노바이러스 단백질이다. 용어 "E4orf1 단백질"은 임의의 아데노바이러스 혈청형으로부터의 E4 유전자에 의해 생성된 E4orf1 단백질을 포함한다. 일례로, 야생형 Ad5 E4orf1 단백질의 아미노산 서열은 본 명세서에서 서열번호 38로 기재되고, PDZ-결합 모티프의 결실을 갖는 변형된 Ad5 E4orf1 단백질은 본 명세서에서 서열번호 39로 기재된다. 또한, 다양한 다른 아데노바이러스 혈청형으로부터의 야생형 E4orf1 단백질 서열이 본 명세서에서 서열번호 55 내지 61로 기재된다. 일부 실시예에서, 변형된 E4orf1 단백질은 서열번호 38, 39 및 55 내지 61 중 어느 하나와 적어도 80%, 적어도 85%, 적어도 90%, 적어도 95%, 적어도 96%, 적어도 97%, 적어도 98%, 또는 적어도 99%의 서열 동일성을 갖는다. 본 명세서에서 고려된 변형된 E4orf1 단백질은 종양세포와 비교하여 정상세포에서 재조합 아데노바이러스의 복제 결함에 기여하는 단백질이다. 본 명세서에 개시된 변형된 E4orf1 단백질은 Ad5 E4orf1 단백질이다. 그러나, 상응하는 변형이 임의의 바람직한 혈청형에서 이루어질 수 있으며, 따라서 본 명세서에 포함된다. 도 28의 E4orf1 정렬에서 나타난 바와 같이, E4orf1 C-말단의 3개 잔기는 아데노바이러스 종 A, B, C, D, E 및 G에서 PDZ-결합 모티프를 구성한다. 일부 실시예에서, 재조합 아데노바이러스는 완전한 결실 E4orf1을 암호화한다.

[0059] E4orf6/7: 아데노바이러스 E4 유전자에 의해 암호화되는 단백질이다. 용어 "E4orf6/7 단백질"은 임의의 아데노바이러스 혈청형으로부터의 E4 유전자에 의해 생산된 E4orf6/7 단백질을 포함한다. 일례로, 야생형 Ad5 E4orf6/7 단백질의 아미노산 서열은 서열번호 40으로 기재되며, 야생형 Ad2 E4orf6/7 단백질의 아미노산 서열은 서열번호 64로 1rwo된다. 일부 실시예에서, E4orf6/7 단백질은 서열번호 40 또는 서열 번호 64 중 어느 하나와 적어도 80%, 적어도 85%, 적어도 90%, 적어도 95%, 적어도 96%, 적어도 97%, 적어도 98%, 또는 적어도 99%의

서열 동일성을 갖는다. 본 명세서에서 고려된 변형된 E4orf6/7 단백질은 종양세포와 비교하여 정상세포에서 재조합 아데노바이러스의 복제 결합에 기여하는 단백질이다. 일부 실시예에서, 변형된 E4orf6/7 단백질은 이의 E2F 결합 부위를 파괴하거나 손상 및/또는 E2F 상호작용을 손상시키는 돌연변이(결실과 같은)를 포함한다. 다른 실시예에서, 변형된 E4orf6/7 단백질은 E2F4의 효율적인 전좌에 필요한 핵 위치 신호가 결실되거나 손상된 변형을 포함한다. E4orf6/7의 예시적인 변형은 하기 섹션에서 더 논의된다.

- [0060] **표피성장인자수용체(EGFR):** 세포의 단백질 리간드의 EGF 패밀리에 속하는 세포표면수용체이다. EGFR은 ErbB-1 및 HER1으로도 알려져 있다. 많은 종류의 암은 EGFR의 과발현을 초래하는 돌연변이를 포함한다.
- [0061] **섬유:** 아데노바이러스 섬유 단백질은 세포표면수용체에 결합을 매개하는 삼량체 단백질이다. 섬유 단백질은 긴 N-말단 샤프트 및 구형 C-말단 노브로 구성된다(도 23A 참조).
- [0062] **FK506 결합 단백질(FKBP):** 단백질 접힘 샤페론으로 기능하는 진핵생물에서 발현되는 단백질 패밀리아이다. FKBP는 라파마이신에 결합하는 능력에 대해 알려져 있다. 예시적인 FKBP 서열은 서열번호 44의 132 내지 238 잔기로서 본 명세서에 기재된다.
- [0063] **FKBP-라파마이신 결합(FRB):** 라파마이신에 결합하는 라파마이신의 포유류 표적(mTOR)의 도메인이다. 예시적인 FRB 서열은 서열번호 42의 547 내지 636 잔기로서 본 명세서에 기재된다. 라파마이신 및 라파로그(rapalog)(AP21967로도 알려짐) 모두에 결합할 수 있는 FRB의 돌연변이 형태(본 명세서의 "FRB*"를 의미함)는 서열번호 43의 547 내지 636 잔기로서 본 명세서에 기재된다. FRB*는 서열번호 43의 620 잔기에 상응하는 인간 mTOR의 2098 위치에서 트레오닌이 류신으로 치환되는 것(T2098L)을 포함한다.
- [0064] **융합 단백질:** 적어도 2개의 다른(이종) 단백질 또는 펩티드의 아미노산 서열을 포함하는 단백질이다. 융합 단백질은 예를 들어, 적어도 2개의 다른(이종) 단백질의 일부를 암호화하는 핵산 서열로부터 조작된 핵산 서열의 발현에 의해 생성될 수 있다. 융합 단백질을 생성하기 위해, 핵산 서열은 동일한 해독틀(reading frame) 내에 있어야 하며, 내부 정지코돈을 포함하지 않아야 한다. 또한, 융합 단백질, 특히 짧은 융합 단백질은 화학합성에 의해 생성될 수 있다.
- [0065] **이종:** 이종 단백질 또는 폴리펩티드는 다른 공급원 또는 종으로부터 유래된 단백질 또는 폴리펩티드를 의미한다.
- [0066] **핵소:** 주요한 아데노바이러스 캡시드 단백질이다. 예시적인 Ad5로부터의 핵소 서열은 본 명세서에서 서열번호 45로 기재된다. E451Q 치환을 포함하는 돌연변이의 핵소 서열은 본 명세서에서 서열번호 46으로 기재된다.
- [0067] **분리된:** "분리된" 생물학적 성분(핵산 분자, 단백질, 바이러스 또는 세포와 같은)은 생물체의 세포 또는 조직에 있는 다른 생물학적 성분, 또는 생물체 자체로부터 실질적으로 분리 또는 정제되어, 다른 염색체 및 염색체 DNA와 RNA, 단백질 및 세포와 같은 성분이 자연적으로 발생한다. "분리된" 핵산 분자 및 단백질은 표준 정제 방법에 의해 정제된 것들을 포함한다. 또한, 상기 용어는 화학적으로 합성된 핵산 분자 및 단백질뿐만 아니라 숙주 세포에서의 재조합 발현에 의해 제조된 핵산 분자 및 단백질을 아우른다.
- [0068] **마이크로 RNA(miRNA 또는 miR):** 식물, 동물 및 바이러스에서 유전자 발현을 조절하는 단일가닥 RNA 분자이다. 마이크로 RNA를 암호화하는 유전자는 전사되어 일차 전사 마이크로 RNA(pri-miRNA)를 형성하고, 이는 전구체 마이크로 RNA(pre-miRNA)라고 불리는 짧은 머리핀(stem-loop) 분자를 형성하며, 이후, 내부핵산가수분해 절단으로 성숙한 마이크로 RNA를 형성한다. 성숙한 마이크로 RNA는 길이가 약 21 내지 23 뉴클레오티드이고, 부분적으로 하나 또는 그 이상의 표적 전령 RNA(mRNA)의 3'UTR에 상보적이다. 마이크로 RNA는 표적 mRNA의 절단을 촉진하거나 세포 전사체의 번역을 차단함으로써 유전자 발현을 조절한다. 본 명세서에 기재된 "간 특이적인 마이크로 RNA"는 간에서만 발현되는 마이크로 RNA, 또는 다른 장기 또는 조직 유형에 비해 간에서 더 많이 발현되는 마이크로 RNA와 같이 간에서 우선적으로 발현되는 마이크로 RNA이다.
- [0069] **변형:** 핵산 또는 단백질 서열의 서열 변화이다. 예를 들어, 아미노산 서열 변형은 예를 들어, 치환, 삽입 및 결실, 또는 이들의 조합을 포함한다. 삽입은 아미노 및/또는 카복실 말단 융합뿐만 아니라 단일 또는 다중 아미노산 잔기의 내부 서열 삽입을 포함한다. 결실은 단백질 서열로부터 하나 또는 그 이상의 아미노산 잔기가 제거됨으로써 특징화된다. 일부 실시예에서, 변형(치환, 삽입 또는 결실과 같은)은 단백질의 특정 활성의 감소 또는 증진과 같은 기능의 변화를 초래한다. 본 명세서에서 사용된 "△" 또는 "델타"는 결실을 의미한다. 예를 들어, E1AΔLXCXE는 LXCXE 모티프의 결실을 갖는 E1A 폴리펩티드를 의미한다. 치환 변형은 적어도 하나의 잔기가 제거되고 다른 잔기가 그 자리에 삽입된 것이다. 아미노산 치환은 전형적으로 단일 잔기이지만, 한번에 다수의 다

른 위치에서 발생할 수 있다. 치환, 결실, 삽입 또는 이의 임의의 조합을 조합하여 최종 돌연변이 서열을 얻을 수 있다. 이러한 변형은 변형을 암호화하는 DNA를 생산함으로써 단백질을 암호화하는 DNA에서 뉴클레오티드의 변형에 의해 일어날 수 있다. 공지된 서열을 갖는 DNA의 미리 결정된 부위에서 삽입, 결실 및 치환 돌연변이를 만드는 기술은 당업계에 잘 알려져 있다. "**변형된**" 단백질, 핵산 또는 바이러스는 상술한 바와 같이 하나 또는 그 이상의 변형을 갖는 것이다.

[0070] **신생물, 악성종양, 암 및 종양**: 신생물은 과도한 세포분열로 인한 조직 또는 세포의 비정상적인 성장이다. 신생물성 증식은 종양을 일으킬 수 있다. 개인의 종양의 양은 종양의 수, 부피 또는 무게로 측정될 수 있는 "종양 부하(tumor burden)"이다. 전이하지 않는 종양을 "양성"이라 한다. 주변 조직을 침범 및/또는 전이할 수 있는 종양을 "악성"이라 한다. 또한, 악성 종양을 "암"이라고 한다.

[0071] 혈액암은 혈액 또는 골수암이다. 혈액학적(또는 혈액성) 암의 예로, 급성 백혈병(예를 들어, 급성 림프구성 백혈병, 급성 골수성 백혈병, 급성 골수아구성 백혈병 및 골수아구성, 전골수성, 골수단핵구성, 단핵구성 및 적백혈병), 만성 백혈병(예를 들어, 만성 골수세포(과립구성) 백혈병, 만성 골수성 백혈병, 및 만성 림프구성 백혈병), 진성 적혈구증가증, 림프종, 호지킨병, 비-호지킨 림프종(저등도(indolent) 및 고등도 형태), 다발성 골수종, 발렌스트롬 마크로글로블린혈증, 중쇄병, 골수이형성증후군, 모발상세포 백혈병 및 골수이형성증을 포함하는 백혈병을 포함한다. 몇몇 경우, 림프종이 고형종양으로 간주된다.

[0072] 고형종양은 일반적으로 낭종이나 액체를 포함하지 않는 비정상적인 조직이다. 고형종양은 양성 또는 악성일 수 있다. 다른 유형의 고형종양은 세포 형태(예를 들어, 육종, 암종 및 림프종)의 유형에 따라 명명된다. 육종 및 암종과 같은 고형종양의 예로, 섬유육종, 점액육종, 지방육종, 연골육종, 골육종 및 기타 육종, 활막종, 증피종, 유방종양, 평활근육종, 횡문근육종, 결장암, 악성림프종, 췌장암, 유방암, 폐암, 난소암, 전립선암, 간세포암종, 편평세포암종, 기저세포암, 선암, 한선암종, 거대종격동전이암, 유두갑상선암종, 크롬친화성세포종피지선암종(pheochromocytomas sebaceous gland carcinoma), 유두암종, 인간유두종바이러스(HPV) 감염된 신생물, 유두선암(papillary adenocarcinomas), 수질암종, 기관지암, 신장암, 간세포암, 담관암, 용모암, 윌름스종양, 자궁경부암, 고환종양, 정상피종, 방광암종, 흑색종, 및 중추신경계종양(예를 들어, 신경교종(예를 들어, 뇌간신경교종 및 혼합된 신경교종), 성상교모세포종(다형성교모세포종이라고도 알려짐), 중추신경계 림프종, 배아세포종, 수모세포종, 슈반종 두개인두종(Schwannoma craniopharyngioma), 상의세포종, 송과체부종양, 혈관모세포종, 청신경종, 핏지교종, 뇌수막종, 신경아세포종, 망막아세포종 및 뇌전이암)를 포함한다.

[0073] **종양 살상 바이러스**: 암/종양세포와 같은 증식성 질환의 세포를 선택적으로 살상시키는 바이러스이다. 암세포의 살상은 생균수를 결정하거나, 암세포에서 세포변성 효과, 세포살상 또는 바이러스 단백질의 합성(예를 들어, 물질대사 표지, 면역블롯, 또는 복제에 필수적인 바이러스 유전자의 RT-PCR), 또는 종양의 크기 감소를 검출하는 것과 같은 당업계에서 확립된 임의의 방법에 의해 검출될 수 있다.

[0074] **작동가능하게 연결된**: 첫번째 핵산 서열은 첫번째 핵산 서열이 두번째 핵산 서열과 기능적인 관계에 놓일 때, 두번째 핵산 서열과 작동가능하게 연결된다. 예를 들어, 프로모터가 코딩 서열의 전사 또는 발현에 영향을 주면 프로모터는 코딩 서열에 작동가능하게 연결된다. 일반적으로, 작동가능하게 연결된 DNA 서열은 연속적이며, 두 개의 단백질 코딩 영역을 합치는 데 필요한 경우 동일한 해독틀에서 결합한다.

[0075] **약학적으로 허용가능한 담체**: 본 명세서에서 유용한 약학적으로 허용가능한 담체(비히클)는 통상적이다. *Repton's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition(1975)는 하나 또는 그 이상의 치료 화합물, 분자 또는 제제(예를 들어, 본 명세서에 개시된 재조합 바이러스)의 약학적인 전달에 적합한 조성물 및 제형을 기술한다.

[0076] 일반적으로, 담체의 성질은 사용되는 특정 투여 방식에 의존할 것이다. 예를 들어, 통상적으로 비경구용 제형은 물, 생리식염수, 평형염류용액, 수성 텍스트로스, 글리세롤 또는 비히클과 같은 약학적으로 및 생리학적으로 허용가능한 유체를 포함하는 주사가능한 유체를 포함한다. 고체 조성물(예를 들어, 분말, 환제, 정제 또는 캡슐 형태)의 경우, 통상의 무독성 고체 담체는 예를 들어, 의료용 만니톨, 락토오스, 전분, 또는 마그네슘 스테아레이트를 포함할 수 있다. 또한, 생물학적으로 중성인 담체, 투여되는 약학적 조성물은 습윤제 또는 유화제, 방부제, 및 pH 완충제 및 예를 들어, 아세트산나트륨 또는 솔비탄 모노라우레이트(sorbitan monolaurate) 등과 같은 소량의 무독성 보조 물질을 포함할 수 있다.

[0077] **폴리펩티드, 펩티드 또는 단백질**: 단량체가 아마이드 결합을 통해 함께 연결된 아미노산 잔기인 중합체이다. 아미노산이 알파-아미노산인 경우, L-광학이성질체 또는 D-광학이성질체가 사용될 수 있다. 용어 "폴리펩티드",

"펩티드" 및 "단백질"은 본 명세서에서 상호교환적으로 사용된다. 이들 용어는 하나 또는 그 이상의 아미노산 잔기가 상응하는 천연 아미노산뿐만 아니라 천연 아미노산 중합체 및 비-천연 아미노산 중합체의 인공적인 화학 모방물인 아미노산 중합체에 적용된다. 용어 "잔기" 또는 "아미노산 잔기"는 단백질, 폴리펩티드, 또는 펩티드에 포함된 아미노산에 대한 언급을 포함한다.

[0078] 폴리펩티드에서의 보존적 치환은 유사한 생화학적 특성을 갖는 다른 아미노산 잔기에 대한 단백질 서열 내의 하나의 아미노산 잔기의 치환이다. 일반적으로, 보존적 치환은 생성된 폴리펩티드의 활성에 거의 또는 전혀 영향을 미치지 않는다. 예를 들어, 하나 또는 그 이상의 보존적 치환(예를 들어, 1, 2, 3, 4 또는 5개 이하의 치환)을 포함하는 단백질 또는 펩티드는 야생형 단백질 또는 펩티드의 구조 및 기능을 유지한다. 폴리펩티드는 예를 들어, 부위 특이적 변이 또는 PCR과 같은 표준절차를 사용하여 폴리펩티드를 암호화하는 뉴클레오타이드 서열을 조작함으로써 하나 또는 그 이상의 보존적 치환을 포함하도록 생성될 수 있다. 일례로, 이러한 변이체는 항체 교차-반응성 또는 면역반응을 유도하는 능력을 시험함으로써 용이하게 선택될 수 있다. 보존적 치환의 예는 하기와 같다.

원래 잔기	보존적 치환
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

[0079]

[0080] 보존적 치환은 일반적으로 (a) 예를 들어, 시트 또는 나선형 형태와 같은 치환 영역에서의 폴리펩티드 골격구조, (b) 표적 부위에서 분자의 전하 또는 소수성, 또는 (c) 결사슬의 부피를 유지한다.

[0081] 일반적으로 단백질 특성에서 가장 큰 변화를 가져올 것으로 예상되는 치환은 예를 들어, (a) 세릴 또는 트레오닐과 같은 친수성 잔기가 류실, 이소류실, 페닐알라닌, 발릴 또는 알라닌과 같은 소수성 잔기로 치환됨; (b) 시스테인 또는 프롤린이 다른 잔기로(또는 이들에 의해) 치환됨; (c) 리실, 아르기닌, 또는 히스타딜과 같은 양전하를 띠는 결사슬을 갖는 잔기가 글루타미드 또는 아스파르틸과 같은 음전하를 띠는 잔기로 치환됨(또는 이들에 의해); 또는 (d) 페닐알라닌과 같은 부피가 큰 결사슬을 갖는 잔기가 글리신과 같은 결사슬을 갖지 않는 잔기로 치환되는 변화인 비-보존적일 것이다.

[0082] **질병의 예방, 치료 또는 개선:** 질병을 "예방"하는 것은 질병의 완전한 발달을 저해하는 것을 의미한다. "치료"는 질병 또는 병적 상태가 발달하기 시작한 후 징후나 증상을 호전시키는 치료적 개입을 의미한다. "개선"은 질병의 징후 또는 증상의 수 또는 중증도 감소를 의미한다.

[0083] **프로모터:** 핵산(예를 들어, 유전자)의 전사를 지시/개시하는 DNA 영역이다. 프로모터는 전사 시작부위 근처에 필요한 핵산 서열을 포함한다. 일반적으로, 프로모터는 그들이 전사하는 유전자 근처에 위치한다. 또한, 프로모터는 선택적으로 먼 인핸서(enhancer) 또는 전사의 개시 위치로부터 수천 염기쌍만큼 위치될 수 있는 리프레서(repressor) 요소를 포함한다. "항시성 프로모터"는 연속적으로 활성이 있으며, 외부 신호 또는 분자에 의해 조절을 받지 않는 프로모터이다. 반면, "유도성 프로모터"의 활성은 외부 신호 또는 분자(예를 들어, 전사인자 또는 테트라사이클린)에 의해 조절된다.

[0084] **단백질 IX(pIX):** 핵산 단백질과 결합하는 아테노바이러스 캡시드의 부수적 인 성분이다.

[0085] **정제된:** 용어 "정제된"은 절대적인 순도를 필요로 하지 않는다; 오히려 이는 상대적 용어를 의미한다. 따라서,

예를 들어, 정제된 펩티드, 단백질, 바이러스, 또는 다른 활성 화합물은 자연적으로 결합된 단백질 및 다른 오염물로부터 전체 또는 부분적으로 분리된 것이다. 특정 실시예에서, 용어 "실질적으로 정제된"은 세포, 세포 배양배지, 또는 다른 미가공 조제물로부터 분리되고, 분획화하여 단백질, 세포 잔해, 및 다른 성분과 같은 초기 조제물의 다양한 성분이 제거된 펩티드, 단백질, 바이러스 또는 다른 활성 화합물을 의미한다.

[0086] **라파마이신**: 면역억제 및 항증식성이 알려진 작은 분자이다. 시롤리무스라고도 알려진 라파마이신은 세균인 스트렙토마이세스 하이그로스코피쿠스(*Streptomyces hygroscopicus*)의 산물로서 처음 발견된 매크로라이드이다. 라파 마이신은 결합하여 mTOR의 활성을 저해한다.

[0087] **Rb-결합세포**: 종양 억제인자 Rb의 수준이 정상 또는 대조군 세포, 또는 Rb 경로가 파괴되거나 비활성인 세포에서의 Rb 수준에 비해 감소된 세포(예를 들어, 종양세포)이다. 용어 "Rb 경로" 또는 "Rb 신호전달경로"는 적어도 부분적으로 pRb/p107, E2F-1/-2/-3, 및 G1 사이클린/cdk 복합체를 포함하는 pRb 활성화에 영향을 미치는 분자를 의미한다. 현재 알려져 있지 않은 분자도 이 정의에 포함될 수 있다.

[0088] **Rb/p16/사이클린 D:cdk4/E2F 복제가 손상되거나 결합된 아데노바이러스**: 세포의 감염시 Rb/p107/p130/p16/E2F/CDK-사이클린 체크 포인트를 포함하는 정상 수준의 기능적인 세포 Rb/p107/p130/p16/사이클린:CDK/E2F 경로 단백질의 존재 하에서 부분적으로 또는 완전히 약화된 복제를 나타내는 아데노바이러스(예를 들어, 변형된/재조합 아데노바이러스)이다. 예를 들어, 감염된 세포가 Rb/p16 경로가 손상되거나 결합된 경우(즉, 감염된 세포가 Rb/p16 경로에서 완전히 기능적인 Rb 또는 다른 단백질을 정상 수준으로 발현하지 못하는 경우), Rb/p16/E2F 경로 복제가 손상된 아데노바이러스의 복제는 정상적으로 진행될 것이다. 반대로, 세포가 정상 수준의 기능적인 Rb(예를 들어, 정상 활성을 갖는 Rb, 본 명세서에서 "Rb 발현세포"라고도 함)를 발현하는 경우, Rb 복제가 손상되거나 결합된 아데노 바이러스의 복제는 약화되거나 방지된다. 세포는 정상 수준의 Rb(예를 들어, Rb 유전자의 조절영역으로의 돌연변이)를 발현하지 못하거나 정상적인 Rb 활성화보다 낮은 돌연변이된 Rb를 발현함으로써 Rb를 손상시키거나 결합시킬 수 있다. 정상 수준의 Rb 및 정상 Rb 활성화 수준은 동일한 유형의 건강한, 질병이 없는 세포에서 발견된다. 따라서, Rb가 손상된 세포는 돌연변이된 Rb 유전자를 포함한다. 선택적으로, Rb가 손상된 세포는 Rb 유전자가 전부 또는 부분적으로 결실된 게놈을 포함한다. Rb가 손상된 세포는 암/종양세포일 수 있다. 정상적인 Rb 기능의 상실을 초래할 수 있는 다른 게놈 병변으로는 CDK 돌연변이, 사이클린 돌연변이 및 증폭, p16 돌연변이 및/또는 후생학적 사일런싱, p107 돌연변이, p130 돌연변이, 및 성장인자 수용체 경로 돌연변이가 있으나, 이에 제한되지 않는다.

[0089] **Rb/p16 종양 억제인자 경로 또는 Rb/p16 경로**: 망막 아세포종(Rb) 단백질, 및 Cdk, E2F, 비정형 단백질 키나아제 C, 및 Skp2를 포함하나, 이에 한정되지 않는 경로에서 다른 단백질/단백질 패밀리를 포함하는 전체 신호전달 경로를 의미한다.

[0090] 용어 "Rb/p16 종양 억제인자 경로가 손상되거나 결합된"은 신호전달경로에서 하나 또는 그 이상의 분자가 손상되거나 결합되었음을 의미한다. 예를 들어, 정상수준 또는 단백질을 발현하지 못하거나 정상적인 활성보다 낮은 활성을 갖는 돌연변이된 단백질을 발현함으로써 경로의 기능이 비정상적으로 된다. 이러한 결합은 유리 E2F의 높은 발현 수준 및 E2F 프로모터의 높은 활성을 초래한다. 따라서, 세포는 정상 수준의 단백질을 발현하지 못하거나 Rb/p16 종양 억제인자 경로에서 정상 이하의 활성을 갖는 돌연변이된 단백질을 발현함으로써 Rb/p16 경로가 손상되거나 결합될 수 있다.

[0091] **재조합**: 재조합 핵산 분자, 단백질 또는 바이러스는 자연적으로 발생하지 않는 서열을 갖는 것이거나 달리 분리된 2개 서열의 인위적인 조합에 의해 만들어진 서열을 갖는다. 이러한 인위적인 조합은 화학적 합성 또는 유전 공학기술에 의한 것과 같이 핵산 분자의 분리된 단편의 인위적인 조작에 의해 달성될 수 있다. 또한, 용어 "재조합"은 천연 핵산 분자, 단백질 또는 바이러스 일부의 첨가, 치환, 또는 결실에 의해서만 변경된 핵산, 단백질 및 바이러스를 포함한다.

[0092] **복제 결합**: 비-종양세포(종양세포에 비해)에서 "복제 결합"을 나타내는 아데노바이러스는 종양세포와 비교하여 정상세포에서 감소된 바이러스 복제를 나타내는 아데노바이러스를 의미한다. 복제 결합은 예를 들어, 바이러스 후기 단백질 발현의 결합, 바이러스 DNA 합성 감소, 감소된 E2F 표적 유전자(예를 들어, 사이클린 A 및 B)로의 유도 능력, 감소된 S기 진입 능력 및/또는 종양세포와 비교하여 정상세포에서 감소된 세포 살상 유도능력으로 입증된다.

[0093] **복제 결합 바이러스**: 주어진 표현형(예를 들어, 조절되지 않은 E2F 경로를 갖는 종양세포)을 갖는 미리 결정된 세포 집단에서 세포 증식을 우선적으로 저해하거나, 세포용해를 유발하거나, 세포살상(총괄하여 살상으로 간주

됨)을 유도하는 바이러스이다. 이러한 바이러스는 세포 증식을 감소 또는 저해하는 능력, 세포 용해를 일으키거나, 세포살상을 유도하거나, 미리 결정된 세포 표현형(예를 들어, 정상, 비-종양세포)을 갖지 않는 세포에서 복제할 수 없거나 제한된다.

- [0094] **서열 동일성:** 둘 또는 그 이상의 핵산 서열, 또는 둘 또는 그 이상의 아미노산 서열 간의 동일성 또는 유사성은 서열 간의 동일성 또는 유사성의 관점에서 표현된다. 서열 동일성은 비율 동일성의 관점에서 측정될 수 있다; 비율이 높을수록 서열이 더 유사하다. 핵산 또는 아미노산 서열의 상동성(homolog) 또는 이종상동성(ortholog)은 표준 방법을 사용하여 정렬될 때 비교적 높은 정도의 서열 동일성/유사성을 갖는다. 이러한 상동성(homology)은 더 관련이 없는 종(예를 들어, 인간 및 *C. elegans* 서열)에 비해 더 밀접한 관련이 있는 종(예를 들어, 인간과 마우스 서열)에서 유래된 이종상동성(orthologous) 단백질이나 cDNA일 때 더 중요하다.
- [0095] 비교를 위한 서열의 정렬 방법은 당업계에 잘 알려져 있다. 다양한 프로그램 및 정렬 알고리즘은 하기에 설명된다: Smith & Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman & Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444, 1988; Higgins & Sharp, *Gene*, 73:237-44, 1988; Higgins & Sharp, *CABIOS* 5:151-3, 1989; Corpet *et al.*, *Nuc. Acids Res.* 16:10881-90, 1988; Huang *et al. Computer Appls. in Biosciences* 8, 155-65, 1992; 및 Pearson *et al.*, *Meth. Mol. Bio.* 24:307-31, 1994. Altschul *et al.*, *J. Mol. Biol.* 215:403-10, 1990은 서열 정렬 방법 및 상동성 계산에 대한 상세한 고려사항을 제시한다.
- [0096] NCBI Basic Local 정렬 Search Tool(BLAST)(Altschul *et al.*, *J. Mol. Biol.* 215:403-10, 1990)는 서열 분석 프로그램인 blastp, blastn, blastx, tblastn 및 tblastx와 관련하여 사용하기 위해 국립생물정보센터(NCBI) 및 인터넷을 포함한 여러 소스에서 사용할 수 있다. 추가적인 정보는 NCBI 웹사이트에서 찾을 수 있다.
- [0097] **혈청형:** 특정 항원에 의해 구별되는 밀접하게 관련된 미생물(예를 들어, 바이러스)의 그룹이다.
- [0098] **개체:** 인간과 인간이 아닌 포유류를 포함하는 범주인 살아있는 다세포 척추동물이다.
- [0099] **합성:** 실험실에서 인위적인 방법에 의해 생산되는 것으로, 예를 들어, 합성 핵산 또는 단백질은 실험실에서 화학적으로 합성될 수 있다.
- [0100] **표적 리간드:** 본 명세서에 기재된 "표적 리간드"는 재조합 아데노바이러스를 표적 리간드에 특이적인 수용체 또는 결합 단백질을 발현하는 특정 세포 유형으로 유도하는 단백질이다. 일부 실시예에서, 표적 리간드는 종양에서 과발현된 세포 표면 단백질(예를 들어, EGFR)에 특이적인 항체이다.
- [0101] **치료제:** 개체에게 적절하게 투여될 때 원하는 치료 또는 예방 효과를 유도할 수 있는 화합물, 소분자, 재조합 바이러스 또는 다른 조성물, 예컨대 안티센스 화합물, 항체, 펩티드 또는 핵산 분자이다. 예를 들어, 암 치료제는 암의 발생 또는 전이를 예방 또는 저해하는 제제를 포함한다.
- [0102] **치료상으로 유효량:** 제제로 치료되는 개체, 또는 세포에서 원하는 효과를 달성하기에 충분한 특정 약제 또는 치료제(예를 들어, 재조합 바이러스)의 양이다. 제제의 유효량은 치료되는 개체 또는 세포 및 치료학적 조성물의 투여 방식을 포함하나, 이에 한정되지 않는 여러 인자에 의존할 수 있다.
- [0103] **U엑손:** 초기 E3 영역과 섬유 유전자(Tollefson *et al.*, *J Virol* 81(23): 12918-12926) 사이의 1 가닥(왼쪽으로 전사)에 위치한 개방형 해독틀이다.
- [0104] **벡터:** 벡터는 숙주세포에서 복제 및/또는 통합하는 벡터의 능력을 손상시키지 않으면서 외래 핵산을 삽입 할 수 있게하는 핵산 분자이다. 벡터는 숙주세포에서 복제기점과 같은 복제를 허용하는 핵산 서열을 포함할 수 있다. 또한, 벡터는 하나 또는 그 이상의 선택 가능한 마커 유전자 및 다른 유전요소를 포함할 수 있다. 발현벡터는 삽입된 유전자 또는 유전자의 전사 및 번역을 허용하기 위해 필요한 조절서열을 포함하는 벡터이다.
- [0105] 달리 설명되지 않는 한, 본 명세서에서 사용되는 모든 기술 및 과학 용어는 본 발명이 속하는 기술 분야의 당업자에 의해 일반적으로 이해되는 것과 동일한 의미를 갖는다. 단수의 용어 "하나(a)", "하나(an)" 및 "그(the)"는 문맥에 달리 명시되어 있지 않는 한 복수의 개체를 포함한다. "A 또는 B를 포함하는"은 A, 또는 B, 또는 A 및 B를 포함하는 것을 의미한다. 핵산 또는 폴리펩티드에 대해 주어진 모든 염기 크기 또는 아미노산 크기, 및 모든 분자량(molecular weight) 또는 분자질량(molecular mass) 값은 근사치이고, 설명을 위해 제공된다. 본 명세서에 기술된 것들과 유사하거나 동등한 방법 및 재료가 본 발명의 실시 또는 테스트에 사용될 수 있지만, 적합한 방법 및 재료는 하기에 기술된다. 본 명세서에 언급된 모든 간행물, 특허출원, 특허 및 기타 참고문헌은 그 전체가 참고문헌으로 인용된다. 상충하는 경우, 용어의 설명을 포함하여 본 명세서가 우선한다. 또한, 재료, 방법

및 실시예는 단지 예시적인 것이며 이에 제한되지 않는다.

[0106] **III. 재조합 아데노바이러스**

[0107] E2F가 조절되지 않은 종양세포에서 선택적으로 복제할 수 있는 재조합 아데노바이러스가 본 발명에 의해 제공된다. 본 명세서에 개시된 재조합 아데노바이러스는 변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 변형된 또는 결실된 E4orf6/7 단백질, 또는 이들의 임의의 조합을 암호화하는 게놈을 갖는다. E1A, E4orf1 및/또는 E4orf6/7 돌연변이의 결과로서, 재조합 아데노바이러스는 정상(즉, 비-종양)세포에서 복제 결함을 나타내지만, 종양세포에서 복제하고 용해시키는 능력을 보유한다. 일 실시예에서, 재조합 아데노바이러스는 동일한 세포 유형(예를 들어, 유방암세포 대 정상유방세포)의 종양세포에 비해 정상(즉, 비-종양)세포에서 적어도 20%, 적어도 40%, 적어도 50%, 적어도 60%, 적어도 70%, 적어도 80%, 적어도 90%, 적어도 95%, 적어도 98%, 또는 적어도 99%의 감소된 복제를 가진다. 예를 들어, 감염된 정상세포 및 감염된 종양세포에서 감염성 입자의 수를 비교함으로써 결정된다.

[0108] 본 발명에서 제공된 재조합 아데노바이러스는 선택적으로 특정 세포 유형을 표적화하고, 간에서 표적화 및 복제를 저해하고, 공통적인 아데노바이러스 혈청형에 대한 기존의 중화 항체를 회피하기 위한 추가적인 변형을 포함한다. 또한, 종양 살상 아데노바이러스를 암호화하는 재조합 핵산 분자가 제공된다.

[0109] EF1 α 프로모터의 조절하에 GFP-루시퍼라제를 발현하는 재조합 리포터 아데노바이러스 유전자 발현벡터가 본 발명에서 추가로 제공된다. 일부 실시예에서, 개시된 리포터 아데노바이러스는 간에서 표적화 및 복제를 저해하는 하나 또는 그 이상의 변형을 포함한다. 또한, 재조합 리포터 아데노바이러스를 암호화하는 재조합 핵산 분자가 제공된다.

[0110] **A. 종양 살상 변형**

[0111] 본 명세서에 개시된 특정 종양 살상 변형은 아데노바이러스 5(Ad5) 게놈 서열을 참조하여 기술된다. 그러나, 임의의 인간 아데노바이러스 혈청형에서 동일한 변형 및 결실이 이루어질 수 있다. 일 실시예에서, 재조합 아데노바이러스는 Ad34이다. 다른 특정 실시예에서, 재조합 아데노바이러스는 Ad11 또는 Ad37이다. 도 27 및 도 28은 인간 아데노바이러스 중 중에서 E1A 및 E4orf1 영역의 서열 유사성을 나타낸다.

[0112] E1A의 CR1 영역은 세포의 E2F에 대한 서열 및 구조적인 상동성을 갖고, E2F-Rb 상호작용과 경쟁한다. 보존된 E1A 소수성 잔기 L43, L46 및 Y47은 Rb와의 상호작용을 위한 소수성 앵커로서 작용한다. L43, L46 및/또는 Y47의 D, E, H, K 또는 R과 같은 극성 아미노산으로의 돌연변이는 E1A-Rb 상호작용을 제거할 것이다. 일부 실시예에서, 재조합 아데노바이러스는 E1A에서 E1A-Rb 상호작용을 파괴하기 위해 Y74H 돌연변이를 포함한다.

[0113] E1A는 LXCXE 모티프를 통해 Rb와 강력하게 상호작용한다. 첫 번째 류신과 중앙 시스테인의 결사슬이 Rb의 B 박스 모티프의 작은 소수성 포켓에 결합하기 때문에, 이 잔기의 결실 또는 작거나(예를 들어, G) 극성 아미노산(예를 들어 D, E, H, K 또는 R)으로의 돌연변이는 E1A-Rb 상호작용을 제거한다. 따라서, 일부 실시예에서, 재조합 아데노바이러스는 E1A에서 LXCXE 모티프(Δ LXCXE) 또는 C124G 치환의 결실을 포함한다.

[0114] E1A 잔기 C124 및 Y47은 모두 Rb에 결합하고, Rb를 불활성화시키는데 중요하다. 따라서, 일부 실시예에서, 재조합 아데노바이러스는 이중 돌연변이 Y/F47H 및 C124G를 암호화하지만, 이들 잔기 또는 부위에 대한 임의의 돌연변이(상술한 바와 같이)는 E1A-Rb 상호작용을 약화시킬 것으로 여겨진다. 따라서, Rb-E1A 상호작용을 파괴시키는 임의의 잔기의 돌연변이 또는 결실이 본 발명에서 고려된다.

[0115] E1A의 잔기 2 내지 11의 결실은 P300/CBP 상호작용을 제거하고, DP-1 상호작용을 방해하고, E2F 표적을 상향조절하는 E1A의 능력을 감소시킨다. 또한, 보존된 R2 또는 H3 잔기의 글리신 또는 알라닌 돌연변이, 또는 다른 아데노바이러스 혈청형으로부터의 소수성 I/L/V4의 극성 잔기 돌연변이(예를 들어, D, E, H, K, 또는 R)와 같은 E1A 2 내지 11 잔기에서의 점 돌연변이가 이 상호작용을 제거하기 위해 본 발명에서 고려된다(도 27의 E1A 정렬 참조).

[0116] E4orf1은 C-말단에서 PDZ-결합 모티프를 통해 PDZ 모티프를 포함하는 세포 단백질과 상호작용한다. 본 명세서에 개시된 일부 실시예에서, E4orf1의 마지막 3개의 아미노산은 이의 PDZ-결합 모티프를 제거하기 위해 결실된다. 또한, V, L, 또는 I와 같은 큰 소수성 잔기에 대한 Ad5 E4orf1의 고도로 보존된 S126의 점 돌연변이; 또는 D, E, H, K, 또는 R과 같은 극성 잔기에 대한 Ad5 E4orf1의 V128의 점 돌연변이가 본 발명에서 고려된다. 이러한 치환은 E4orf1의 PDZ-결합 활성을 제거하고, E4orf1의 이러한 형질전환 기능을 제거하는 것으로 여겨진다. E4orf1에서의 동일한 결실 및/또는 돌연변이는 Ad34와 같은 다른 아데노바이러스 혈청형에서 만들어질 수 있다.

또한, E4orf1의 완전한 결실이 개시된다.

- [0117] 본 발명은 재조합 아데노바이러스의 게놈이 변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 변형된 또는 결실된 E4orf6/7 단백질, 또는 이들의 임의의 조합을 암호화하는 재조합 아데노바이러스를 제공하며, 상기 재조합 아데노바이러스는 정상세포에 비해 종양세포에서 복제 결함을 나타낸다.
- [0118] 일부 실시예에서, 게놈은 변형된 E1A 단백질 및 변형된 또는 결실된 E4orf1 단백질을 암호화한다. 다른 실시예에서, 게놈은 변형된 E1A 단백질 및 결실된 E4orf6/7 단백질을 암호화한다. 또 다른 실시예에서, 게놈은 변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질을 암호화한다.
- [0119] 일부 실시예에서, 재조합 아데노바이러스의 변형된 E1A 단백질은 LXCXE 모티프의 결실; 2 내지 11 잔기의 결실; C124G 치환; Y47H 치환; Y47H 치환 및 C124G 치환; 또는 Y47H 치환, C124G 치환 및 2 내지 11 잔기의 결실을 포함한다. 이러한 돌연변이는 서열번호 32의 아미노산 서열을 갖는 Ad5 E1A 이다; 그러나, E1A 서열이 고도로 보존되기 때문에 임의의 다른 인간 아데노바이러스에서 상응하는 돌연변이가 만들어질 수 있다(도 27 참조).
- [0120] 일부 실시예에서, 변형된 E4orf1 단백질은 PDZ-결합 모티프의 결실을 포함한다. 다른 실시예에서, E4orf1 단백질은 C-말단의 10개 아미노산 또는 20개 아미노산의 결실, 또는 D68의 A, K, R, P, F, G 또는 L로의 돌연변이를 포함한다.
- [0121] 일부 실시예에서, 변형된 E4orf6/7 단백질은 이의 E2F 결합부위를 파괴 또는 손상 및/또는 E2F 상호작용을 손상시키는 돌연변이(예를 들어, 결실)를 포함한다. 다른 실시예에서, 변형된 E4orf6/7 단백질은 E2F4의 효율적인 전좌에 필요한 핵 위치 신호를 삭제하거나 손상시키는 변형을 포함한다.
- [0122] 특히 제한하지 않는 예로서, 재조합 아데노바이러스의 게놈은 LXCXE 모티프의 결실을 포함하는 변형된 E1A 단백질 및 결실된 E4orf6/7 단백질을 암호화한다(예를 들어, AdSyn-C0181, AdSyn-C0312, AdSyn-C0313, AdSyn-C0335, AdSyn-C0442 참조);
- [0123] LXCXE 모티프의 결실을 포함하는 변형된 E1A 단백질, PDZ-결합 모티프의 결실을 포함하는 변형된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0285 참조);
- [0124] LXCXE 모티프의 결실을 포함하는 변형된 E1A 단백질, 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0286 참조);
- [0125] C124G 치환을 포함하는 변형된 E1A 단백질 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0287 참조);
- [0126] C124G 치환을 포함하는 변형된 E1A 단백질, PDZ-결합 모티프의 결실을 포함하는 변형된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0288 참조);
- [0127] C124G 치환을 포함하는 변형된 E1A 단백질, 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0289 참조);
- [0128] 2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0290 참조);
- [0129] 2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질, PDZ-결합 모티프의 결실을 포함하는 변형된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0291 참조);
- [0130] 2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질, 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0292 참조);
- [0131] Y47H 치환 및 C124G 치환을 포함하는 변형된 E1A 단백질, 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0293 참조);
- [0132] Y47H 치환 및 C124G 치환을 포함하는 변형된 E1A 단백질, PDZ-결합 모티프의 결실을 포함하는 변형된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0294 참조);
- [0133] Y47H 치환 및 C124G 치환을 포함하는 변형된 E1A 단백질, 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0295 참조);
- [0134] Y47H 치환, C124G 치환 및 2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질, 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0296 참조);

- [0135] Y47H 치환, C124G 치환 및 2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질, PDZ-결합 모터프의 결실을 포함하는 변형된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질(예를 들어, AdSyn-C0297); 또는
- [0136] Y47H 치환, C124G 치환 및 2 내지 11 잔기의 결실을 포함하는 변형된 E1A 단백질, 결실된 E4orf1 단백질, 및 결실된 E4orf6/7 단백질을 포함하는 변형된 E1A 단백질 (예를 들어, AdSyn-C0298 참조).
- [0137] 특정 실시예에서, 재조합 아데노바이러스의 게놈은 서열번호 6 내지 8, 10 내지 12, 14, 15, 18 내지 20, 22 내지 26, 30, 31 및 82 내지 87 중 임의의 하나와 적어도 90%, 적어도 95%, 적어도 96%, 적어도 97%, 적어도 98% 또는 적어도 99%의 상동성을 갖거나, 재조합 아데노바이러스의 게놈은 서열번호 6 내지 8, 10 내지 12, 14, 15, 18 내지 20, 22 내지 26, 30, 31, 82 내지 87, 및 92 내지 97 중 임의의 하나를 포함하거나 구성하고,
- [0138] 같은 세포 유형(예를 들어, 유방암세포 vs. 정상유방세포)의 종양세포에 비해 정상(즉, 비-종양)세포에서 적어도 20%, 적어도 40%, 적어도 50%, 적어도 60%, 적어도 70%, 적어도 80%, 적어도 90%, 적어도 95%, 적어도 98%, 또는 적어도 99% 감소된 복제를 갖는다. 예를 들어, 정상세포와 종양세포에서 감염성 입자의 수를 비교함으로써 결정된다.
- [0139] 일부 실시예에서, 재조합 아데노바이러스의 게놈은 E3-RID α /RID β 및 E3-14.7k 코딩서열의 결실을 포함한다.
- [0140] **B. 간 비표적화 변형**
- [0141] 본 명세서에 개시된 재조합 아데노바이러스는 간에서 바이러스를 비표적화하는 변형을 추가로 포함할 수 있다. Ad5 핵손은 혈중에서 X 인자에 결합할 수 있으며, 간에서 쿠퍼(Kupffer) 세포에 의한 흡수를 유도하여 전신성 전파를 예방하고 염증을 제한할 수 있다. 이를 극복하기 위해, 재조합 아데노바이러스는 E1에서의 추가적인 게놈 변형 및 간에서의 흡수 및 발현을 방지하는 코어 모듈을 포함하도록 조작되었다.
- [0142] 이러한 추가적인 변형을 포함하는 게놈 모듈을 시험하고, 생체 내 사용 및 유전자 전달을 위한 Ad5 발현백터를 생성하기 위해 E1A/E1 유전자가 결실되었고 EF1 α 유도된 루시페라제-GFP 융합체로 대체되었다. 정맥 내 투여시, AdSyn-C0199는 루시페라제 생체발광에 의해 입증되는 바와 같이 간에서 주로 축적되었다(도 22 참조).
- [0143] 간에서의 표적 발현을 방지하기 위해, 바이러스는 간에서 특이적으로 발현되는 마이크로 RNA에 대한 E1A의 3'UTR 결합부위를 포함하도록 조작되었다. 특정 실시예에서, miR122는 이의 발현 및 결합부위가 인간 및 마우스의 간세포에서 보존되므로 간 특이적인 마이크로 RNA로 선택되었다. 일부 실시예에서, 간 특이적인 miR122에 대한 2개의 마이크로-RNA 결합부위는 바이러스 유전자 발현 및 세포 염증반응을 유도하는 간에서의 잔류 바이러스 흡수를 방지하기 위해 E1A의 3'UTR에 삽입되었다. Adsyn-C0199와 달리, AdSyn-C0200(하기 도 22 및 실시예 2 참조)은 간에서 루시페라제 발현을 유도하지 않는다.
- [0144] X 인자에 결합하는 Ad5 핵손을 통한 간에서의 바이러스 흡수 및 격리를 막기 위해, 바이러스는 간 흡수를 막는 핵손(E451Q)에서 추가적인 돌연변이로 조작되었다. AdSyn199 및 Adsyn200과 달리, AdSyn-C0171은 간에 축적되지 않고 대신 다른 기관, 이 경우에는 비-종양을 가진 동물의 비장 및 림프절을 표적으로 할 수 있다(도 22).
- [0145] E1A, E4orf1 및/또는 E4orf6/7에서 하나 또는 그 이상의 돌연변이를 갖는 E2F 선택성 바이러스의 전신성 전달을 가능하게 하기 위해, 재조합 아데노바이러스는 핵손에서 간을 비표적화하는 E451Q 변형을 추가로 포함하도록 조작되었다(예를 들어, AdSyn-C0335 및 AdSyn-C0442 참조; 도 20 및 21 및 실시예 2 참조).
- [0146] 아데노바이러스 핵손 유전자에 대한 다른 돌연변이는 간에서의 아데노바이러스 축적을 방지하기 위해 본 발명에서 고려된다. 예를 들어, 재조합 아데노바이러스는 핵손의 9개의 초가변영역을 다른 혈청형의 영역으로 대체함으로써 간에서 비표적화될 수 있다.
- [0147] 본 발명은 재조합 아데노바이러스의 게놈이 변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 변형된 또는 결실된 E4orf6/7 단백질, 또는 이들의 임의의 조합을 암호화하고, 추가로 변형된 핵손 단백질을 암호화하며, 종양세포와 비교하여 정상세포에서 복제 결함을 나타내는 재조합 아데노바이러스를 제공한다(예를 들어, AdSyn-C0335 참조).
- [0148] 일부 실시예에서, 변형된 핵손 단백질은 E451Q 치환을 포함한다. 특정 실시예에서, 재조합 아데노바이러스의 게놈은 서열번호 30, 서열번호 92 또는 서열번호 97과 적어도 90%, 적어도 95%, 적어도 96%, 적어도 97%, 적어도 98% 또는 적어도 99% 상동성을 갖거나, 게놈은 서열번호 30, 서열번호 92 또는 서열번호 97의 뉴클레오타이드 서열을 포함하거나 상기 서열로 구성된다.
- [0149] 또한, 본 발명은 재조합 아데노바이러스의 게놈이 변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 변형

된 또는 결실된 E4orf6/7 단백질, 또는 이들의 임의의 조합을 암호화하고, 간 특이적인 마이크로 RNA를 위한 하나 또는 그 이상의 결합부위를 추가로 포함하며, 종양세포와 비교하여 정상세포에서 복제 결합을 나타내는 재조합 아데노바이러스를 제공한다.

[0150] 일부 실시예에서, 간 특이적인 마이크로 RNA를 위한 하나 또는 그 이상의 결합 부위는 E1A의 3'-UTR에 위치된다. 일부 실시예에서, 간 특이적인 마이크로 RNA는 miR-122, miR-30 또는 miR-192이다.

[0151] C. 유도성 재표적화를 위한 변형

[0152] 본 명세서에 개시된 재조합 아데노바이러스 종양세포 수용체에 대한 생체 내 유도성 재표적화(inducible retargeting)를 위해 추가로 변형될 수 있다. 흔히 사용되는 Ad2/5 벡터는 감염을 위해 상피세포에 존재하지만, 대부분의 전이성 암에는 없는 족사키 아데노바이러스 수용체(CAR)에 의존한다. "RapAD"는 융합 단백질을 통해 임의의 종양세포 수용체(또는 임의의 세포 유형)로의 아데노바이러스 감염의 라파마이신/라파로그 유도성 표적화를 가능하게 한다(본 명세서에서 전체가 참고 문헌으로 인용되는 PCT 출원번호 WO 2013/138505 참조). 유연한 어댑터 시스템의 사용은 바이러스 캡시드의 조립을 방해하지 않는다. 이상적인 재표적화 융합 단백질은 특정 암 세포 표면 분자에 대해 강한 친화성을 갖는 항체이다. 낙타과 및 상어는 단일 10kDa 가변 VHH 도메인을 통해 에피토프를 인식하는 단일-도메인 항체(sdAb)를 암호화한다. 합성생물학을 이용하면 폐암에서 고도로 상향조절된 EGFR 및 CEACAM5와 같은 특정 분자를 인식하는 VHH가 만들어질 수 있다. 라파마이신은 CAR이 하향조절된 전이성 종양세포에서 EGFR에 대한 합성 아데노바이러스 감염을 재표적화한다는 것이 증명되었다.

[0153] 조작된 아데노바이러스는 생물학적으로 직교하는(orthogonal) 라파로그 AP21967로 표적화될 수 있다. 라파마이신은 종양 살상 아데노바이러스 치료와 합리적인 조합이지만, 라파마이신에 의한 세포 및 유기체의 영향을 피하는 것이 바람직할 수 있는 경우가 있을 수 있다. 따라서, 아데노바이러스 재표적화를 위한 또 다른 옵션으로, 라파마이신의 구조상 동족체인 AP21967이 사용될 수 있다. AP21967은 FKBP 및 돌연변이 FRB* 도메인(mTOR 돌연변이 T2098L)과 안정한 이중이량체를 형성할 수 있지만 야생형 FRB 도메인과는 형성할 수 없다. 라파마이신과 달리, AP21967은 표준 하류 기질인 p70S6 키나아제 인산화에 의해 입증되는 것 처럼, mTOR 신호전달을 불활성화하지 않는다. 그러나 AP21967은 여전히 FKBP-VHH 융합체를 통해 종양세포에 바이러스를 발현하는 FRB*-섬유(FRB-섬유는 제외)의 재표적화를 유도할 수 있다(도 18).

[0154] EGFR-표적화된 아데노바이러스가 돌연변이 FRB 도메인(FRB*)과 함께 변형될 때, 이는 라파마이신 및 AP21967과 더불어 EGFR을 통해 세포를 감염시키도록 유도될 수 있다(도 18 내지 21 및 하기 실시예 2 참조).

[0155] 본 발명은 게놈이 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 변형된 또는 결실된 E4orf6/7 단백질, 또는 이들의 임의의 조합을 암호화하고, FKBP 단백질에 융합된 표적화 리간드를 추가로 암호화하며, 야생형 FRB 단백질 또는 T2098L 치환을 포함하는 돌연변이 FRB 단백질에 융합된 아데노바이러스 섬유단백질을 암호화하는 재조합 아데노바이러스를 제공한다.

[0156] 일부 실시예에서, 표적화 리간드는 예컨대 낙타과 VHH 항체와 같은 단일 도메인 항체이다. 특정 실시예에서, 단일 도메인 항체는 EGFR, 또는 종양세포에서 상향조절되는 다른 분자에 특이적이다. 비-제한적인 실시예에서, 재조합 아데노바이러스의 게놈은 서열 번호 25, 서열번호 26 또는 서열번호 88에 대해 적어도 90%, 적어도 95%, 적어도 96%, 적어도 97%, 적어도 98% 또는 적어도 99%의 상동성을 갖거나, 서열번호 25, 서열번호 26 또는 서열번호 88의 뉴클레오타이드 서열을 포함하거나 상기 서열로 구성된다(예를 들어, AdSyn-C0312, AdSyn-C0313 및 AdSyn-C0205 참조).

[0157] 일부 실시예에서, FKBP-표적화 리간드 융합 단백질은 E3-RID α /RID β 및 E3-14.7k 코딩서열을 결실시킴으로써 아데노바이러스 게놈에 수용된다. 그러나, 아데노바이러스 게놈의 다른 위치가 고려된다. 예를 들어, 표적화 리간드-FKBP 융합 단백질은 또한 자가-절단 P2A 링커서열(예를 들어, AdSyn-C0440; 서열번호 68 참조)을 갖는 E3-ADP에 N-말단 융합체 또는 자가-절단 P2A 서열을 갖는 E1B-55k에 C-말단 융합체로서 삽입될 수 있다.

[0158] 또한, 게놈이 변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 변형된 또는 결실된 E4orf6/7 단백질, 또는 이들의 임의의 조합을 암호화하고, 추가로 T2098L 치환을 포함하는 돌연변이 FRB 단백질에 융합된 아데노바이러스 섬유단백질을 암호화하는 재조합 아데노바이러스를 제공한다. 일부 실시예에서, 게놈은 E451Q 치환을 포함하는 변형된 핵소 단백질질을 추가로 암호화한다. 특정 실시예에서, 재조합 아데노바이러스의 게놈은 서열번호 31과 적어도 90%, 적어도 95%, 적어도 96%, 적어도 97%, 적어도 98% 또는 적어도 99% 상동성을 갖거나 서열번호 31의 뉴클레오타이드 서열을 포함하거나 상기 서열로 구성된다(예를 들어, AdSyn-C0336 참조).

- [0159] 전술한 유도성 재표적화된 재조합 아데노바이러스는 돌연변이 핵소 단백질(예를 들어, E451Q 치환)과 같은 간 재표적화 변형을 추가로 포함할 수 있다. 택일적으로 또는 추가적으로, 재조합 아데노바이러스의 게놈은 간 특이적인 마이크로 RNA에 대한 하나 또는 그 이상의 결합 부위를 포함할 수 있다(전술한 바와 같다).
- [0160] **D. 재표적화를 위한 키메라 섬유 단백질**
- [0161] Ad5 및 다른 많은 혈청형의 섬유 단백질이 세포 부착을 위해 콕사키 아데노바이러스 수용체(CAR)에 결합하는 것으로 밝혀졌지만, 다른 혈청형은 CD46, 데스모 글레인 2, 시알산 등을 사용하는 것으로 알려져 있다. 따라서, 본 발명에서는 키메라 섬유 단백질을 암호화하는 게놈을 갖는 재조합 아데노바이러스가 제공되고, 이를 통해 재조합 바이러스를 다른 세포 수용체로 표적화한다(도 23A 내지 23E 및 하기 실시예 3 참조).
- [0162] 본 발명은 게놈이 변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 변형된 또는 결실된 E4orf6/7 단백질, 또는 이들의 임의의 조합을 암호화하고, 추가로 키메라 섬유 단백질을 암호화하는 재조합 아데노 바이러스를 제공한다.
- [0163] 일부 실시예에서, 키메라 섬유 단백질은 제1 아데노바이러스 혈청형으로부터의 섬유 샤프트(shaft) 및 제2 아데노바이러스 혈청형으로부터의 섬유 노브(knob)를 포함한다. 일부 실시예에서, 제1 아데노바이러스 혈청형은 Ad5 이고, 제2 아데노바이러스 혈청형은 Ad3, Ad9, Ad11, Ad12, Ad34 또는 Ad37이다. 하나의 비-제한적인 실시예에서, 제1 아데노바이러스 혈청형은 Ad5이고, 제2 아데노바이러스 혈청형은 Ad34이다.
- [0164] 특정 실시예에서, 재조합 아데노바이러스의 게놈은 서열번호 82, 서열번호 83 또는 서열번호 84의 뉴클레오타이드 서열을 포함한다.
- [0165] 키메라 섬유를 발현하는 재조합 종양 살상 아데노바이러스는 전술한 바와 같이 유도성 표적화 및/또는 간 재표적화 변형을 추가로 포함할 수 있다.
- [0166] **E. 중화 항체를 피하기 위한 캡시드 교환**
- [0167] 본 발명은 천연 아데노바이러스 모듈화(modularity)를 이용하여 기존의 중화 항체를 피할 수 있는 키메라 바이러스를 생성하는 것을 추가로 고려한다. 특히, 완전한 '캡시드' 모듈 교환(거의 게놈의 60%)을 가지며 기존의 항체에 보이지 않게하고 반복된 접종을 가능하게 하는 Ad5 기반 바이러스가 본 명세서에 개시되어있다. 캡시드 교환된 재조합 아데노바이러스는 실시예 4에 추가로 나타나있다(도 24 내지 도 26 참조).
- [0168] 본 발명은 게놈이 변형된 E1A 단백질, 변형된 또는 결실된 E4orf1 단백질, 변형된 또는 결실된 E4orf6/7 단백질, 또는 이들의 임의의 조합을 암호화하고, 캡시드-교환된 키메라 아데노바이러스를 암호화하는 재조합 아데노바이러스를 제공한다. 일부 실시예에서, 게놈의 E1, E3 및 E4 영역은 제1 아데노바이러스 혈청형으로부터 유도되고 게놈의 E2B, L1, L2, L3, E2A 및 L4 영역은 제2 아데노바이러스 혈청형으로부터 유래된다. 일부 실시예에서, 제1 아데노바이러스 혈청형의 E1 영역은 제2 아데노바이러스 혈청형으로부터의 pIX 단백질을 암호화하도록 변형; 및/또는 제1 아데노바이러스 혈청형의 E3 영역은 제2 아데노바이러스 혈청형으로부터 U엑손 및 섬유 단백질을 암호화하도록 변형된다. 특정 실시예에서, 제1 아데노바이러스 혈청형은 Ad5이고 제2 아데노바이러스 혈청형은 Ad3, Ad9, Ad11, Ad34 또는 Ad37이다.
- [0169] 특정 실시예에서, 재조합 아데노바이러스의 게놈은 서열번호 85의 뉴클레오타이드 서열, 또는 서열번호 92 내지 97 중 임의의 어느 하나를 포함한다.
- [0170] 재조합 종양 살상, 캡시드-교환된 아데노바이러스는 상술한 바와 같이 키메라 섬유, 유도성 표적화 및/또는 간 재표적화 변형을 추가로 포함할 수 있다.
- [0171] **F. 다른 변형**
- [0172] 본 명세서에 개시된 재조합 아데노바이러스는 또한 종양을 선택적으로 증진시키고, 특정 세포 유형으로의 감염을 지시하고, 존재하는 중화 항체를 피하는 것과 같은 추가의 변형을 포함할 수 있다.
- [0173] 일 실시예에서, 재조합 아데노바이러스는 인테그린-결합 RGD 모티프에서 RGE로의 돌연변이를 갖는 캡시드 단백질 펜톤과 함께 E1, E3, 및 E4 돌연변이를 갖는 EGFR-표적화된 종양 살상 아데노바이러스이다(예를 들어, AdSyn-C0511; 서열번호 86). 상기 펜톤 돌연변이는 비장에서 바이러스 흡수를 감소시키고 항바이러스 면역반응을 약화시키는 것으로 나타났다.
- [0174] 다른 실시예에서, 재조합 종양 살상 아데노바이러스는 섬유 단백질에서 RGD 펩티드의 삽입과 함께 E1 및 E4 돌

연변이를 포함한다(예를 들어, AdSyn-C0512; 서열번호 87). 섬유 단백질에서 RGD 삽입은 혈관 내피세포를 포함하는 다양한 종류의 세포로의 감염을 극적으로 증가시키는 것으로 나타났다.

[0175] **G. 재조합 리포터 아데노바이러스**

[0176] 본 발명은 재조합 리포터 아데노바이러스를 제공한다. 일부 실시예에서, 재조합 아데노바이러스의 게놈은 E1A의 결실을 포함하고 EF1 α -루시퍼라제를 암호화한다(예를 들어, AdSyn-C0199, AdSyn-C0200 및 AdSyn-C0171 참조).

[0177] 일부 실시예에서, 재조합 아데노바이러스의 게놈은 간 특이적인 마이크로 RNA에 대한 하나 또는 그 이상의 결합 부위를 추가로 포함 및/또는 변형된 핵산 단백질을 암호화한다. 일부 실시예에서, 간 특이적인 마이크로 RNA에 대한 하나 또는 그 이상의 결합 부위는 E1A의 3'-UTR에 위치한다. 특정한 비-제한적인 실시예에서, 간 특이적인 마이크로 RNA는 miR-122이다. 일부 실시예에서, 변형된 핵산 단백질은 E451Q 치환을 포함한다.

[0178] 특정한 비-제한적인 실시예에서, 재조합 아데노바이러스의 게놈은 적어도 90%, 적어도 95%, 적어도 96%, 적어도 97%, 적어도 98% 또는 적어도 99% 상동성을 갖거나 서열번호 27(AdSyn-C0199), 서열번호 28(AdSyn-C0200), 또는 서열번호 29(AdSyn-C0171)의 뉴클레오티드 서열을 포함하거나 상기 서열로 구성된다.

[0179] **IV. 야생형과 돌연변이 바이러스 서열**

[0180] 본 명세서에 개시된 재조합 아데노바이러스의 재조합 아데노바이러스 게놈이 본 발명에 의해 제공된다. 특히, 서열번호 1 내지 31 및 68 내지 98 중 임의의 어느 하나와 적어도 90%, 적어도 95%, 적어도 96%, 적어도 97%, 적어도 98% 또는 적어도 99% 상동성을 갖는 뉴클레오티드 서열을 포함하는 재조합 핵산 분자가 제공된다. 특정 실시예에서, 재조합 핵산은 서열번호 1 내지 31 및 68 내지 98 중 임의의 어느 하나의 뉴클레오티드 서열을 포함하거나 상기 서열로 구성된다.

[0181] 또한, 재조합 아데노바이러스 게놈을 포함하는 벡터가 제공된다. 일부 실시예에서, 서열번호 1 내지 31 및 68 내지 98 중 임의의 하나와 적어도 90%, 적어도 95%, 적어도 96%, 적어도 97%, 적어도 98% 또는 적어도 99% 상동성을 갖는 핵산 분자를 포함하는 벡터가 제공된다. 일부 실시예에서, 서열번호 1 내지 31 및 68 내지 98 중 임의의 어느 하나의 뉴클레오티드 서열을 포함하거나 상기 서열로 구성된 핵산 분자를 포함하는 벡터가 제공된다.

[0182] 하기에 개시된 재조합 아데노바이러스에 의해 발현되는 야생형 및 돌연변이 아데노바이러스 단백질 서열이 제공된다.

[0183] **E1A 돌연변이**

[0184] 하기 E1A 서열에서 LXCXE 모티프는 밑줄로 표시된다. 이 모티프는 Ad5 E1A(서열번호 32)의 아미노산 122 내지 126에 존재한다. Y47H 및 C124G 치환은 굵게 표시하였다.

[0185] **Ad5 E1A**

[0186] MRHIICHGGVITEEMAASLLDQLIEEVLADNLPPPSHFEPPTLHELYDLDTAPEDPNEEAVSQIFPDSVMLAVQEGIDLLTFPPAPGSPEPPHLSRQPEQP
EQRALGPVSMPLNVEVIDLT**CH**EGFPPSDDDEEGEEFVLDYVEHPGHGCRSCHYHRRNTGDPDIMCSLCYMRTCGMFVYSPVSEPEPEPEPEPARPT
RRPKMAPAILRRPTSPVSRECNSSDSCDSGPSNTTPEIHPVVPLCPKPVAVRVGGRRQAVECIEDLLNEPGQPLDLCKRPRP(서열번호 32)

[0187] **Ad5 E1A Δ LXCXE**

[0188] MRHIICHGGVITEEMAASLLDQLIEEVLADNLPPPSHFEPPTLHELYDLDTAPEDPNEEAVSQIFPDSVMLAVQEGIDLLTFPPAPGSPEPPHLSRQPEQP
EQRALGPVSMPLNVEVIDAGFPSSDDEEGEEFVLDYVEHPGHGCRSCHYHRRNTGDPDIMCSLCYMRTCGMFVYSPVSEPEPEPEPEPEPARPTRPKM
APAILRRPTSPVSRECNSSDSCDSGPSNTTPEIHPVVPLCPKPVAVRVGGRRQAVECIEDLLNEPGQPLDLCKRPRP(서열번호 33)

[0189] **Ad5 E1A C124G**

[0190] MRHIICHGGVITEEMAASLLDQLIEEVLADNLPPPSHFEPPTLHELYDLDTAPEDPNEEAVSQIFPDSVMLAVQEGIDLLTFPPAPGSPEPPHLSRQPEQP
EQRALGPVSMPLNVEVIDLT**CH**EGFPPSDDDEEGEEFVLDYVEHPGHGCRSCHYHRRNTGDPDIMCSLCYMRTCGMFVYSPVSEPEPEPEPEPEPARPT
RRPKMAPAILRRPTSPVSRECNSSDSCDSGPSNTTPEIHPVVPLCPKPVAVRVGGRRQAVECIEDLLNEPGQPLDLCKRPRP(서열번호 34)

[0191] **Ad5 E1A Δ 2-11**

[0192] MEEMAASLLDQLIEEVLADNLPPPSHFEPPTLHELYDLDTAPEDPNEEAVSQIFPDSVMLAVQEGIDLLTFPPAPGSPEPPHLSRQPEQPEQRALGPVSMPLNVEVIDLT**CH**EGFPPSDDDEEGEEFVLDYVEHPGHGCRSCHYHRRNTGDPDIMCSLCYMRTCGMFVYSPVSEPEPEPEPEPEPARPTRPKMAPAILRRPTSPVSRECNSSDSCDSGPSNTTPEIHPVVPLCPKPVAVRVGGRRQAVECIEDLLNEPGQPLDLCKRPRP(서열번호 35)

- [0193] **Ad5 E1A Y47H C124G**
- [0194] MRHI ICHGGVITEEMAASLLDQLIEEVLADNLPPPSHFEPPTLHELHDLDTAPEDPNEEAVSQIFPDSVMLAVQEGIDLLTFPPAPGSPEPPHLSRQPEQP
EQRALGPVSMPLVPEVIDLTGHEAGFPSSDDEDEEGEEFVLDYVEHPGHGCRSCHYHRRNTGDPDIMCSLCYMRTCGMFVYSPVSEPEPEPEPEPEPARPT
RRPKMAPAILRRPTSPVSRECNSSDSCDSGPSNTPEIHPVVPLCPKIPVAVRVGGRRQAVECIEDLLNEPGQPLDLCKRPRP(서열번호 36)
- [0195] **Ad5 E1A Δ 2-11 Y47H C124G**
- [0196] MEEMAASLLDQLIEEVLADNLPPPSHFEPPTLHELHDLDTAPEDPNEEAVSQIFPDSVMLAVQEGIDLLTFPPAPGSPEPPHLSRQPEQPEQALGPVSMPL
NLVPEVIDLTGHEAGFPSSDDEDEEGEEFVLDYVEHPGHGCRSCHYHRRNTGDPDIMCSLCYMRTCGMFVYSPVSEPEPEPEPEPEPEPARPTRRPKMAPAILR
RPTSPVSRECNSSDSCDSGPSNTPEIHPVVPLCPKIPVAVRVGGRRQAVECIEDLLNEPGQPLDLCKRPRP(서열번호 37)
- [0197] **Ad5 E4orf1 및 E4orf6/7**
- [0198] Ad5 E4orf1의 C-말단(서열번호 38의 126 내지 128 잔기)에서 PDZ-결합 모티프는 밑줄로 표시된다.
- [0199] **Ad5 E4orf1**
- [0200] MAAAVEALYVVLREGAILPRQEGFSGVYVFFSPINFVIPPMAV_mSLRLRVCIPPGYFGRFLALTDVNQPDVFTESYIMTPDMTEELSVVLFNHGDQFFY
GHAGMAVVRL_mIRVVFPPVVRQASNV(서열번호 38)
- [0201] **Ad5 E4orf1 Δ PDZb**
- [0202] MAAAVEALYVVLREGAILPRQEGFSGVYVFFSPINFVIPPMAV_mSLRLRVCIPPGYFGRFLALTDVNQPDVFTESYIMTPDMTEELSVVLFNHGDQFFY
GHAGMAVVRL_mIRVVFPPVVRQA(서열번호 39)
- [0203] **Ad5 E4orf6/7**
- [0204] MTTSGVPFGMTLRPTRSRLSRTPYSRDLPPFETETRATILEDHPLLPECNTLTMHNAWTSPSPPV
- [0205] KQPQVGQPVAAQLDSDMNLSELPGEFINITDERLARQETVWNI TPKNMSVTHDMMLFKASRGERTVYSVCWEG
- [0206] GGRLNTRVL(서열번호 40)
- [0207] E2F와의 결합을 제거 또는 손상시키거나, 핵 위치 신호를 결실시키거나 손상시키는 변형된 E4orf6/7 단백질은 암호화하는 게놈을 갖는 재조합 아데노바이러스가 본 발명에서 고려된다. 일부 실시예에서, 변형된 E4orf6/7 단백질은 E2F 결합 부위를 결실/손상시키기 위해 C-말단에 약 60개, 약 50개, 약 40개, 약 30개, 약 20개 또는 약 10개 아미노산의 결실을 포함한다. 다른 실시예에서, E4orf6/7 단백질은 C-말단 10개의 아미노산에서 결실, 틀 이동(frameshift) 또는 삽입, 또는 E2F 결합을 제거하거나 손상시키는 단백질의 C-말단 세번째로부터 33개 아미노산의 결실을 포함한다. 일부 실시예에서, 돌연변이는 E2F 상호작용을 손상시키는 81 내지 91개의 아미노산을 포함한다.
- [0208] 다른 실시예에서, 변형된 E4orf6/7 단백질은 E2F4의 효율적인 전좌를 위해 필요한 핵 위치 서열을 제거하기 위해 58개 아미노산의 N-말단 결실을 포함한다. Ad2 및 Ad5 E4orf6/7의 13 및 38번째 아미노산 사이에는 8개의 아르기닌 잔기가 있으며, 이는 이 영역에 대해 25% 이상의 아르기닌 함량을 가진 것과 동일하다. E4orf6의 N-말단에 있는 아르기닌 잔기의 전체 클러스터링은 다른 아데노바이러스 혈청형에서 유지된다. 알라닌(또는 이 영역을 통해 핵 위치를 제거하기 위한 다른 적절한 잔기)에 대해 16, 18, 21, 22, 27 및/또는 29 아르기닌 잔기를 치환하는 돌연변이가 고려된다.
- [0209] 다른 실시예에서, 변형된 E4orf6/7 단백질은 E2F 이중 부위 점유를 유도하기 위한 E4orf6/7의 능력을 제거 또는 저해하기 위한 하나 또는 그 이상의 변형을 포함한다. 특정한 비-제한적인 실시예에서, 프롤린, 알라닌, 리신, 아스파르트산 또는 글루탐산에 대한 F125의 돌연변이; 또는 P, A, K, R, G, F에 대한 D121의 돌연변이를 포함한다.
- [0210] 다른 E4orf6/7 돌연변이는 T133A, R101A, Q105P 또는 E2F 단일 부위 점유를 막는 임의의 돌연변이; 알파나선을 파괴하고 E2F 결합을 막는 M84N 또는 P, G, K, L, H 및/또는 E93A, 또는 K, P, G, R, L, M을 포함한다. 고려되는 다른 돌연변이는 T133Q 또는 A, K, G, P, L, H; G141L, P, K, H, F, A; 또는 V149N, K, P, H, G, E, D를 포함한다.
- [0211] **섭유 서열**

- [0212] 하기 재조합 섬유 서열에서, FRB 서열은 밑줄로 표시된다. FRB*에 있는 돌연변이는 굵게 표시된다.
- [0213] **Ad5 섬유**
- [0214] MKRARPSSEDTFNPVYPYDTETGPPTVPFLTPPFVSPNGFQESPPGVLSRLSEPLVTSNGMLALKMGNGLSLDEAGNLTSQNVTTVPPLKKTCSNINLEIS
APLTVTSEALTVAAPLMVAGNTLTMQSQAPLTVHDSKLSIATQGPLTVSEGLALQTSGLTTTDSSTLTITASPPLTTATGSLGIDLKEPIYTQNGKLG
LKYGAPLHVTDLNTLTVATGPGVTINNTSLQTKVTGALGFDSQGNMQLNVAGGLRIDSQNRRLILDVSYPFDAQNQLNRLGQGPLFINSAHNLDINYNKG
LYLFTASNNKKLEVNLSAKGLMFDATAIAINAGDGLFEGSPNAPNTNPLKTKIGHGLEFDSNKAMVPLGTGLSFDSTGAI TVGNKNNDKLTWTTTPAPS
PNCRLNAEKDAKLTVLTKCGSQILATVSVLAVKGLAPI SGTVQSAHL I IRFDENGVLNNSFLDPEYWNFRNGDLTEGTAYTNAVGFMPNLSAYPKSHGK
TAKSNIVSQVYLNGDKTKPVTLTITLNGTQETGDTTPSAYSMSFSWDWSGHNYINEIFATSSYTF SYIAQE(서열번호 41)
- [0215] **Ad5 FRB-섬유**
- [0216] MKRARPSSEDTFNPVYPYDTETGPPTVPFLTPPFVSPNGFQESPPGVLSRLSEPLVTSNGMLALKMGNGLSLDEAGNLTSQNVTTVPPLKKTCSNINLEIS
APLTVTSEALTVAAPLMVAGNTLTMQSQAPLTVHDSKLSIATQGPLTVSEGLALQTSGLTTTDSSTLTITASPPLTTATGSLGIDLKEPIYTQNGKLG
LKYGAPLHVTDLNTLTVATGPGVTINNTSLQTKVTGALGFDSQGNMQLNVAGGLRIDSQNRRLILDVSYPFDAQNQLNRLGQGPLFINSAHNLDINYNKG
LYLFTASNNKKLEVNLSAKGLMFDATAIAINAGDGLFEGSPNAPNTNPLKTKIGHGLEFDSNKAMVPLGTGLSFDSTGAI TVGNKNNDKLTWTTTPAPS
PNCRLNAEKDAKLTVLTKCGSQILATVSVLAVKGLAPI SGTVQSAHL I IRFDENGVLNNSFLDPEYWNFRNGDLTEGTAYTNAVGFMPNLSAYPKSHGK
TAKSNIVSQVYLNGDKTKPVTLTITLNGTQETGDTTEMWHEGLEEASRLYFGERNVKGMEVLEPLHAMMERGPQTLKETSFNQAYGRDLM EAQEWCRKYMK
SGNVKDLTQAWDLYYHVFRRI SKQPSAYSMSFSWDWSGHNYINEIFATSSYTF SYIAQE(서열번호 42)
- [0217] **Ad5 FRB* -섬유**
- [0218] MKRARPSSEDTFNPVYPYDTETGPPTVPFLTPPFVSPNGFQESPPGVLSRLSEPLVTSNGMLALKMGNGLSLDEAGNLTSQNVTTVPPLKKTCSNINLEIS
APLTVTSEALTVAAPLMVAGNTLTMQSQAPLTVHDSKLSIATQGPLTVSEGLALQTSGLTTTDSSTLTITASPPLTTATGSLGIDLKEPIYTQNGKLG
LKYGAPLHVTDLNTLTVATGPGVTINNTSLQTKVTGALGFDSQGNMQLNVAGGLRIDSQNRRLILDVSYPFDAQNQLNRLGQGPLFINSAHNLDINYNKG
LYLFTASNNKKLEVNLSAKGLMFDATAIAINAGDGLFEGSPNAPNTNPLKTKIGHGLEFDSNKAMVPLGTGLSFDSTGAI TVGNKNNDKLTWTTTPAPS
PNCRLNAEKDAKLTVLTKCGSQILATVSVLAVKGLAPI SGTVQSAHL I IRFDENGVLNNSFLDPEYWNFRNGDLTEGTAYTNAVGFMPNLSAYPKSHGK
TAKSNIVSQVYLNGDKTKPVTLTITLNGTQETGDTTEMWHEGLEEASRLYFGERNVKGMEVLEPLHAMMERGPQTLKETSFNQAYGRDLM EAQEWCRKYMK
SGNVKDLLQAWDLYYHVFRRI SKQPSAYSMSFSWDWSGHNYINEIFATSSYTF SYIAQE(서열번호 43)
- [0219] **표적화 리간드 서열**
- [0220] **EGFRVHH-GS-FKBP**
- [0221] MAVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWRQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNKNTVDLQMNSLRPEDTAIYYCAAAAG
STWYGTLYEYDYWGQGTQVTVSSGSGSGSTGVQVETISPGDGRTPFKRGQTCVVHYTGMLDGGKFDSSSRDRNKPFFKMLGKQEVIRGWEEGVAQMSVGQRA
KLITSPDYAYGATGHPGIIPPHATLVFDVELLKL(서열번호 44; FKBP12 서열은 밑줄로 표시된다)
- [0222] **핵손 서열**
- [0223] **Ad5 핵손**
- [0224] MATPSMMPQWSYMHISGQDASEYLSPLVQFARATETYFSLNNKFRNPTVAPTHDVTDRSQRLTLRFIPVDREDTAYSYKARFTLAVGDNVRVDMASTYFD
IRGVLDRGPTFKPYSGTAYNALAPKGAPNPCEWDEAATALEINLEEEEDDNEDEVEQAEQQKTHVFGQAPYSGINITKEGIQIGVEGQTPKYADKTFQPEP
QIGESQWYETEINHAAGRVLKKTPMKPCYGSYAKPTNENGQGI L VKQNGKLESQVEMQFFSTTEATAGNGDNLTPKVLYSEDVDIETPDTHISYMP TI
KEGNSRELMGQQSMPNPNYIAFRDNF IGLMYNSTGNMGLAGQASQLNAVVDLQDRNTELSYQLLLDSIGDRTRYFSMWNQAVDSYDPDVRI IENHGTED
ELPNYCFPLGGVINTETLTKVKPKTGQENGWEKDATEFSDKNEIRVGNNFAMEINLNANLWRNFLYSNIALYLPDKLKYSNSNVKISDNPNTYDYMNKRVA
PGLVDCYINLGARWSLDYMDNVNPFNHHRNAGLRYRSMLLGNRGYVPFHIQVPQKFFAIKNLLLLPGSYTYEWNFRKDVNMVQLSSLGNDLRVDGASIKFDS
ICLYATFFPMAHNTASTLEAMLRNDTNDQSFNDYLSAANMLYIPANATNVPISIPSRNWAAFRGWAFRLKTKETPSLGSYDPPYYSIGSI PYLDGTFY
NHTFKKVAITFDSSVSWPGNDRLLTPNEFEIKRSVDGEGYNVAQCMMTKDWFLVQMLANYNIGYQGFYIPESYKDRMYSFFRNFQPMQRVVD DTKYKDYQ
VGILHQHNNSGFVGLAPTMREGQAYPANFPYPLIGKTAVDSITQKKFLCDRTLWRIPFSSNFMSMGALTDLGQNLLYANSAHALDMTFEVDPMDEPTLLYV
LFEVFDVVRVHRPHRGV IETVYLRTPFSAGNATT(서열번호 45)
- [0225] **Ad5 핵손 E451Q**
- [0226] MATPSMMPQWSYMHISGQDASEYLSPLVQFARATETYFSLNNKFRNPTVAPTHDVTDRSQRLTLRFIPVDREDTAYSYKARFTLAVGDNVRVDMASTYFD
IRGVLDRGPTFKPYSGTAYNALAPKGAPNPCEWDEAATALEINLEEEEDDNEDEVEQAEQQKTHVFGQAPYSGINITKEGIQIGVEGQTPKYADKTFQPEP

QIGESQWYETEINHAAGRVLKKTTPMKPCYGSYAKPTNENGGQILVKQQNGKLESQVEMQFFSTTEATAGNDNLTPKVVLYSEDVDIETPDTHISYMP
KEGNSRELMGQQSMPNRPNYIAFRDNF IGLMYNSTGNMGVLAGQASQLNAVVDLQDRNTELSYQLLLDSIGDRTRYFSMNQAVDSYDPDVRI IENHGTED
ELPNYCFPLGGVINTETLTKVKPKTGQENGWEKDATEFSDKNQIRVGNFAMEINLNANLWRNFLYSNIALYLPDKLKYSPSNVKISDNPNYDYMKNRVVA
PGLVDCYINLGARWSLDYMDNVNPFNHHRNAGLRYSMLLGNGRYVPFHIQVPQKFFAIKNLLLPGSYTYEWNFRKDVNMVLQSSLGNDLRVDGASIKFDS
ICLYATFFPMAHNTASTLEAMLRNDTNDQSFNDYLSAANMLYPIPANATNPVISIPSRNWAAFRGWAFTRLKTKETPSLGSYDPPYYTSGSIPYLDGTFYL
NHTFKKVAITFDSSVSWPGNDRLLTPNEFEIKRSVDGEGYNVAQCNMTKDWFLVQMLANYNIGYQGFYIPESYKDRMYSFFRNFPQMSRQVDDTKYKDYQQ
VGILHQHNSGFVGYLAPTMREGQAYPANFPYPLIGKTAVDSITQKKFLCDRTLWRIPFSSNFMSMGALTDLGQNLLYANSAHALDMTFEVDPMDEPTLLYV
LFEVFDVVRVHRPHRGV IETVYLRTPF SAGNATT(서열번호 46; E451Q 치환은 굵게 표시된다)

[0227] **E3 서열**

[0228] **Ad5 E3-RID α**

[0229] MIPRVFILLTLVALFCACSTLAASHIEVDCIPAFYVYLLYGFVTLTLICSLITVVIQICIDWVCFAYLRHHPQYRDRTIAELLRL(서열번호 65)

[0230] **Ad5 E3-RID β**

[0231] MKFTVTFLLIICLSAFCSPTSKPQRHISCRFTRIWNIPSCYNEKSDLSEAWLYAIISVMVFCSTILALAIYPYLDIGWNAIDAMNHPTFPAPAm ϕ PLQQVV
AGGFVPANQPRPPSPTPTEISYFNLTGGDD(서열번호 66)

[0232] **Ad5 E3-14.7K**

[0233] MTDLDLEMDGIITEQRLLERRAAAEQQRMNQELQDMVNLHQCKRGIFCLVKQAKVYDSNTTGHR

[0234] LSYKLPTKRQKLVVMGKPIITITQHSVETEGCIHSPCQGPEDLCTLIKTLCLGLKDLIPFN(서열번호 67)

[0235] **V. 약학적 조성물**

[0236] 본 발명은 재조합 아데노바이러스(또는 재조합 아데노바이러스를 암호화하는 하나 또는 그 이상의 핵산 또는 벡터)를 포함하는 조성물을 제공한다. 상기 조성물은 시험관내 또는 생체내에서의 임의의 제형 및 투여에 적합하다. 선택적으로, 조성물은 제공된 제제 및 약학적으로 허용가능한 담체 중 하나 또는 그 이상을 포함한다. 적합한 담체 및 이의 제형은 *Remington: The Science and Practice of Pharmacy, 22nd Edition*, Loyd V. Allen *et al.*, editors, Pharmaceutical Press(2012)에 기재되어있다. 약학적으로 허용가능한 담체는 생물학적으로 또는 다른 방식으로 바람직하지 않은 물질을 포함하지 않는다. 즉, 물질은 바람직하지 않은 생물학적 효과를 일으키지 않거나 포함되어 있는 제약 조성물의 다른 성분과 유해한 방식으로 상호작용하지 않고 개체에게 투여된다. 개체에게 투여되는 경우, 담체는 활성성분의 분해를 최소화하고 개체에서의 부작용을 최소화하도록 임의로 선택된다.

[0237] 재조합 바이러스(또는 재조합 아데노 바이러스를 암호화하는 하나 또는 그 이상의 핵산 또는 벡터)는 정맥내 투여, 예를 들어 볼루스(bolus)와 같은 공지된 방법에 따라, 또는 일정 기간에 걸친 연속적인 주입에 의해, 근육내, 복강내, 뇌척수내, 피하, 관절내, 활액막내, 척추강내, 구강, 국소, 종양내 또는 흡입경로로 투여될 수 있다. 투여는 국소적이거나 전신적일 수 있다. 조성물은 국소적인 투여, 경구 투여, 비경구 투여, 정맥내 투여, 관절내 투여, 복강내 투여, 근육내 투여, 피하 투여, 병변내 투여, 경피 투여, 간내 투여, 두개내 투여를 포함하는 임의의 여러가지 투여 방법, 분무/흡입, 또는 기관지 내시경을 통한 설치에 의해 투여될 수 있다. 따라서, 조성물은 국소적인 치료 또는 전신적인 치료가 바람직할지의 여부 및 치료될 영역에 따라 여러가지 방법으로 투여된다.

[0238] 일부 실시예에서, 투여를 위한 조성물은 약학적으로 허용가능한 담체, 바람직하게는 수성 담체에 용해된 본 명세서에 기재된 재조합 아데노바이러스(또는 재조합 게놈)를 포함할 것이다. 다양한 수성 담체, 예를 들어 완충 식염수 등이 사용될 수 있다. 이러한 용액은 멸균 처리되어 있으며, 일반적으로 바람직하지 않은 물질이 없다. 이러한 조성물은 통상의 잘 알려진 멸균 기술에 의해 멸균될 수 있다. 조성물은 예를 들어, 아세트산나트륨, 염화나트륨, 염화칼륨, 염화칼슘, 락트산나트륨 등과 같은 pH 조절제 및 완충제, 독성 조절제 등과 같은 생리적인 조건을 유사하게 하는데 필요한 약학적으로 허용가능한 보조물질을 함유할 수 있다. 이들 중화성체제의 농도는 다양할 수 있으며, 선택된 특정 투여방식 및 대상의 요구에 따라 주로 체액의 부피, 점도, 체중 등에 기초하여 선택될 것이다.

[0239] 약학적 제형, 특히 재조합 바이러스의 약학적 배합물은 원하는 순도를 갖는 재조합 아데노바이러스(또는 재조합

아데노바이러스를 암호화하는 하나 또는 그 이상의 핵산)를 임의의 약학적으로 허용가능한 담체, 부형제 또는 안정화제와 혼합함으로써 제조될 수 있다. 이러한 제형은 동결건조된 제형 또는 수용액일 수 있다.

[0240] 허용가능한 담체, 부형제, 또는 안정화제는 사용된 용량과 농도가 수혜자에게 독성을 나타내지 않는다. 허용가능한 담체, 부형제, 또는 안정화제는 아세트산염, 인산염, 시트르산염, 및 기타 유기산; 항산화제(예를 들어, 아스코르브산)방부제, 저분자량 폴리펩티드; 혈청 알부민 또는 젤라틴과 같은 단백질, 또는 폴리비닐피롤리돈과 같은 친수성 중합체; 및 글루코스, 만노오스, 또는 텍스트린을 포함하는 아미노산, 단당류, 이당류, 및 다른 탄수화물; 킬레이트제; 및 이온성 및 비이온성 계면활성제(예를 들어, 폴리소르베이트); 나트륨과 같은 염-형성 반대-이온; 금속착물(예를 들어, Zn-단백질 복합체); 및/또는 비이온성 계면활성제일 수 있다. 재조합 아데노바이러스(또는 재조합 아데노바이러스를 암호화하는 하나 또는 그 이상의 핵산)는 임의의 적절한 농도의 감염 단위로 제형화될 수 있다.

[0241] 경구 투여에 적합한 제형은 (a) 물, 식염수 또는 PEG 400과 같은 희석제에 현탁된 재조합 아데노바이러스의 유효량과 같은 액체 용액; (b) 각각 미리 결정된 양의 활성성분을 액체, 고형물, 과립 또는 젤라틴으로서 포함하는 캡슐, 봉지 또는 정제; (c) 적합한 액체에서의 현탁액; 및 (d) 적합한 에멀전으로 구성될 수 있다. 정제 형태는 하나 또는 그 이상의 락토오스, 수크로오스, 만니톨, 솔비톨, 인산칼슘, 옥수수전분, 감자전분, 결정셀룰로오스(microcrystalline cellulose), 젤라틴, 콜로이드성 이산화규소, 활석, 마그네슘 스테아레이트, 스테아르산, 및 기타 부형제, 착색제, 충전제, 결합제, 희석제, 완충제, 습윤제, 방부제, 향료, 염료, 봉해제, 및 약학적으로 호환성이 있는 담체를 포함할 수 있다. 사탕 형태는 예를 들어, 수크로오스뿐만 아니라 젤라틴 및 글리세린 또는 수크로오스 및 아카시아 에멀전, 젤 등과 같은 비활성 염기에 활성성분을 포함하는 사탕과 같은 활성성분을 향료내에 포함할 수 있으며, 추가로 활성성분, 당분야에 공지된 담체를 포함할 수 있다.

[0242] 단독으로 또는 다른 적합한 성분과 조합된 재조합 아데노바이러스(또는 재조합 아데노바이러스를 암호화하는 하나 또는 그 이상의 핵산)는 흡입을 통해 투여될 수 있는 에어로졸 제형으로 제조될 수 있다(즉, 이들은 "분무될 수 있다"). 에어로졸 제형은 디클로로디플루오로메탄, 프로판, 질소 등과 같은 가압된 허용가능한 추진제에 배치될 수 있다.

[0243] 예를 들어, 관절내(관절내), 정맥내, 근육내, 종양내, 피내, 복강내 및 피하 경로와 같은 비경구 투여에 적합한 제형은 항산화제, 완충제, 정균제, 및 제형을 의도된 수용자의 혈액과 등장성으로 만드는 용액을 포함할 수 있는 수성 및 비수성의 등장성 멸균 주사 용액, 및 현탁제, 용해제, 증점제, 안정화제 및 방부제를 포함할 수 있는 수성 및 비수성 멸균 현탁액을 포함한다. 제공된 방법에서, 조성물은, 예를 들면, 정맥 내 주입, 경구적으로, 국소적으로, 복강 내(intraperitoneally), 방광내(intravesically), 종양내(intratumorally), 또는 척수강내(intrathecally)로 투여될 수 있다. 비경구용 투여, 종양내 투여, 및 정맥 내 투여는 투여의 바람직한 방법이다. 화합물의 제형은 앰플 및 바이알과 같은, 단위-용량 또는 다중-용량의 봉인된 용기에서 존재할 수 있다.

[0244] 주사 용액 및 현탁액은 이전에 설명된 종류의 멸균 분말, 과립제, 및 정제로부터 제조될 수 있다. 생체 외 치료를 위해 아데노바이러스에 의해 형질도입 또는 감염되거나 또는 핵산으로 형질감염된 세포는 상기에서 설명된 바와 같이 정맥 내 또는 비경구용으로 투여될 수 있다.

[0245] 약학적 제제는 바람직하게는 단위 복용량(unit dosage) 형태이다. 이러한 형태에서 제제는 유효 성분의 적절한 용량을 포함하는 단위 용량(unit dose)으로 세분화된다. 따라서, 약학적 조성물은 투여 방법에 의존하는 다양한 단위 복용량 형태로 투여될 수 있다. 예를 들면, 경구 투여에 적절한 단위 복용량 형태는 분말, 정제, 환제, 캡슐 및 캔디를 포함하나, 이에 한정하지 않는다.

[0246] 일부 실시예에서, 조성물은 다른 세포내 수용체에 결합하는 재조합 아데노바이러스와 같은, 적어도 2개의 다른 재조합 아데노바이러스를 포함한다. 예를 들면, 조성물에서 재조합 아데노바이러스의 적어도 하나는 키메라 섬유 단백질을 발현할 수 있다. 대안적으로, 재조합 아데노바이러스는 조성물 내의 바이러스 간의 상이한 표적 리간드와 FKBP 융합 단백질을 암호화하여 다른 세포내 수용체를 표적화할 수 있다. 일부 실시예에서, 조성물은 2개, 3개, 4개, 5개 또는 6개의 다른 재조합 아데노바이러스를 포함한다.

[0247] VI. 치료 방법

[0248] 본 명세서에 개시된 재조합 아데노바이러스 조성물은 치료적 또는 예방적 치료에 투여될 수 있다. 특히, 개체에서 종양 세포 생존능을 저해하는, 개체에서 종양 진행을 저해하는, 개체에서 종양 부피를 감소하는 및/또는 개체에서 암을 치료하는 방법을 제공한다. 상기 방법은 개체에게 재조합 아데노바이러스(또는 이의 조성물)의 치

료학적 유효량의 투여를 포함한다. 전문에 걸쳐 설명된 바와 같이, 아데노바이러스 또는 약학적 조성물은 정맥 내, 혈관 내, 척수강 내, 근육 내, 피하 내, 복강 내(intraperitoneally), 또는 경구를 포함하는 여러 가지 방법으로 투여되나, 제한되지 않는다. 선택적으로, 상기 방법은 개체에게 하나 이상의 추가 치료제를 투여하는 것을 포함한다. 일부 실시예에서, 상기 치료제는 화학치료제이다. 다른 실시예에서, 치료제는 면역 조절제이다. 또 다른 실시예에서, 치료제는 CDK 4 저해제와 같은, CDK 저해제이다.

[0249] 일부 실시예에서, 암 또는 종양은 폐, 전립선, 직장(colorectal), 유방, 갑상선, 신장, 또는 간의 암 또는 종양이거나, 또는 백혈병 유형이다. 일부 경우에서, 암은 전이성이다. 일부 실시예에서, 종양은 유방, 뇌하수체(pituitary), 갑상선, 또는 전립샘(prostate gland); 뇌, 간, 뇌척수막, 골, 난소, 자궁, 또는 자궁경부의 종양; 단핵구성 또는 골수성 백혈병; 선암, 샘종(adenoma), 별아교세포종(astrocytoma), 방광종양(bladder tumor), 뇌종양, 버킷림프종(Burkitt's lymphoma), 유방암종, 경부암종, 결장암종, 신장암종, 간 암종, 폐 암종, 난소 암종, 췌장 암종(pancreatic carcinoma), 전립선 암종, 직장 암종, 피부 암종, 위암종, 고환암종, 갑상선 암종, 연골육종, 융모암, 섬유종, 섬유육종, 교모세포종, 신경교종, 간세포암, 조직구종(histiocytoma), 평활세포근모종(leiomyoblastoma), 평활근육종, 림프종, 지방육종 세포, 유방 종양, 수모세포종, 골수종(myeloma), 형질세포종(plasmacytoma), 신경아세포종, 신경교종(neuroglioma), 골육종(osteogenic sarcoma), 췌장종양(pancreatic tumor), 뇌하수체 종양(pituitary tumor), 망막아세포종, 횡문근육종, 육종(sarcoma), 고환종양, 가슴샘종(thymoma), 또는 윌름스종양(Wilms tumor)이다. 종양은 뇌, 신경, 눈, 및 뇌척수막(별아교세포종(astrocytomas), 신경교종, 교모세포종, 망막아세포종, 신경종(neuromas), 신경아세포종, 신경초종(Schwannomas), 및 수막종(meningiomas)을 포함하여)의 혈관종(hemangiomas), 흑색종, 육종(카포시 육종(Kaposi's sarcoma)뿐만 아니라 골 및 연조직으로부터 발생하는 것을 포함하여) 및 종양뿐만 아니라, 유방, 결장, 직장(rectum), 폐, 구강인두(oropharynx), 후두인두(hypopharynx), 식도(esophagus), 위(stomach), 췌장(pancreas), 간, 쓸개(gallbladder) 및 쓸개관(bile ducts), 소장(small intestine), 요로(urinary tract)(신장, 방광 및 요로상피를 포함하여), 여성생식관(암컷 genital tract), (자궁경부, 자궁, 및 난소 뿐만 아니라 융모암 및 임신영양막병(gestational trophoblastic)을 포함하여), 남성생식관(전립선, 정낭(seminal vesicles), 고환 및 생식 세포 종양을 포함하는), 내분비샘(endocrine glands)(갑상선, 부신(adrenal), 및 뇌하수체(pituitary glands)를 포함하는), 및 피부의 암종(carcinoma)을 포함하는 원발 및 전이성 고형종양 모두를 포함한다. 일부 측면에서, 림프종(호지킨(Hodgkin's) 및 비-호지킨 림프종 모두)의 치료에서 뿐만 아니라 백혈병과 같은 혈액암(hematopoietic malignancies)(즉, 녹색증(chloromas), 형질세포종(plasmacytomas) 및 균상식육종(mycosis fungoides) 및 피부 T-세포 림프종/백혈병의 플라크 및 종양)으로부터 발생하는 고형종양은 치료될 수 있다. 추가적으로, 치료제는 본 명세서에서 설명된 종양으로부터의 전이 방지에 사용할 수 있다.

[0250] 치료적 적용에서, 재조합 아데노바이러스 또는 이의 조성물을 개체에게 치료학적 유효량 또는 용량을 투여할 수 있다. 상기 사용을 위한 유효량은 질병의 심각성 및 환자 건강의 통상적인 상태에 의존할 것이다. 조성물의 단일 또는 다중 투여는 환자의 필요성 및 내약성에 따라 복용량 및 빈도에 의존적으로 투여될 수 있다. “환자” 또는 “개체”는 인간 및 다른 동물, 특히 포유동물 모두를 포함한다. 따라서, 상기 방법은 인간 치료 및 수의학 적용 모두에 적용 가능하다.

[0251] 변형된 서열을 가지는 아데노바이러스의 유효량은 각 개체별로 결정되고, 적어도 부분적으로, 사용된 특정한 재조합 아데노바이러스에 기반한다; 개인의 크기, 연령, 성별; 및 증식 세포의 크기 및 다른 특성. 예를 들면, 인간 치료를 위하여, 재조합 바이러스의 적어도 10^3 (플라크 형성단위(plaque forming units, PFU))가 사용되고, 예를 들면, 적어도 10^4 , 적어도 10^5 , 적어도 10^6 , 적어도 10^7 , 적어도 10^8 , 적어도 10^9 , 적어도 10^{10} , 적어도 10^{11} , 또는 적어도 10^{12} PFU 바이러스가 사용되고, 예를 들면 증식 세포의 유형, 크기 및 수 또는 신생물(neoplasm)의 존재에 의존하여 재조합 바이러스의 대략 10^3 내지 10^{12} PFU가 사용된다. 유효량은 약 1.0 pfu/kg 체중부터 약 10^{15} pfu/kg 체중까지(예를 들어, 약 10^2 pfu/kg 체중부터 약 10^{13} pfu/kg 체중까지)일 수 있다. 재조합 아데노바이러스는 단일 용량 또는 다중 용량(예를 들어, 2, 3, 4, 6, 또는 그 이상의 용량)으로 투여될 수 있다. 다중 용량은 동시에 또는 연속적으로(예를 들어, 일 또는 주(weeks)의 기간에 걸쳐) 투여될 수 있다.

[0252] 일부 실시예에서, 상기 제공된 방법은 개체에게 항암제 또는 다른 치료적 처치(종양의 외과 절제와 같은)와 같은, 하나 이상의 추가 치료제를 투여하는 것을 포함한다. 대표적인 항암제들은 유사분열 저해제(mitotic inhibitor), 알킬화제, 항-대사물질, 삽입성 항생제, 성장 인자 저해제, 세포주기 저해제, 효소, 국소이성화효소(topoisomerase) 저해제, 항-생존제, 생물학적 반응조절제, 항-호르몬(예를 들어 항-안드로겐(항-

androgens)), 항혈관생성 제 및 CDK 저해제와 같은, 화학치료제를 포함하나, 이에 한정되지 않는다. 다른 항암 치료는 방사선 치료 및 암세포를 특이적으로 표적하는 다른 항체를 포함한다.

- [0253] 알킬화제의 비제한적인 예들은 질소 머스타드(nitrogen mustards)(메클로르에타민(mechlorethamine), 사이클로포스포아마이드, 멜팔란(melphalan), 우라실 머스타드(uracil mustard) 또는 클로람부실(chlorambucil)과 같은), 알킬 설포네이트(alkyl sulfonates)(부설판(Busulfan)과 같은), 니트로소우레아(nitrosoureas)(카르무스틴(carmustine), 로무스틴(lomustine), 세무스틴(semustine), 스트렙토조신(streptozocin), 또는 다카바진(dacarbazine)과 같은)를 포함한다.
- [0254] 항대사물질의 비제한적인 예들은 엽산 유사체 (메토크렉사트(methotrexate)와 같은), 피리미딘(pyrimidine) 유사체(5-FU 또는 시타라빈(cytarabine)과 같은), 및 머캅토피린(mercaptopurine) 또는 티오구아닌(thioguanine)과 같은 퓨린(purine) 유사체를 포함한다.
- [0255] 천연물의 비제한적인 예들은 빈카 알칼로이드(vinca alkaloids)(빈블라스틴(vinblastine), 비크리스틴(vincristine) 또는 빈데신(vindesine)과 같은), 에피포도필로톡신(epipodophyllotoxins)(에토포사이드(etoposide) 또는 테니포사이드(teniposide)와 같은), 항생제(닥티노마이신(dactinomycin), 다우노루비신(daunorubicin), 독소루비신(doxorubicin), 블레노마이신(bleomycin), 필라카마이신(plicamycin), 또는 미토마이신 C(mitomycin C)와 같은), 및 효소 (L-아스파라기나제(L-asparaginase)와 같은)를 포함한다.
- [0256] 다양한 제제의 비제한적인 예들은 플래티늄배위결합복합체(platinum coordination complexes) (또한 시스플라틴(cisplatin)으로 알려진 시스-디아민-디클로플래티늄 II(cis-diamine-dichloroplatinum II)와 같은), 치환된 우레아 (하이드록시우레아(hydroxyurea)와 같은), 메틸 하이드라진(methyl hydrazine) 유도체(프로카바진(procarbazine)와 같은), 및 부신피질 억제제(adrenocortical suppressants) (마이토테인(mitotane) 및 아미노글루테티미드(aminogluthetamide)와 같은)를 포함한다.
- [0257] 호르몬 및 길항제의 비제한적인 예들은 아드레노코르티코(adrenocorticosteroids)(프레드니손(prednisone)와 같은), 프로제스틴(progestins)(하이드록시프로제스테론 카프로에이트(hydroxyprogesterone caproate), 메드록시프로제스테론 아세트산염(medroxyprogesterone acetate), 및 메그에스트롤 아세트산염(magestrol acetate)과 같은), 에스트로젠(estrogens)(디에틸stil베스트롤(diethylstilbestrol) 및 에티닐 에스트라디올(ethinyl estradiol)와 같은), 안티에스트로젠(antiestrogens)(타목시펜(tamoxifen)와 같은), 및 안드로젠(androgens)(테스테론 프로피오네이트(testosterone propionate) 및 플루옥시메스테론(fluxymesterone)와 같은)을 포함한다.
- [0258] 가장 통상적으로 사용된 화학요법 약물의 예들은 아드리아마이신(Adriamycin), 알케란(Alkeran), 아라-C(Ara-C), BiCNU, 부설판(Busulfan), CCNU, 카르보플라티늄(Carboplatinum), 시스플라티늄(Cisplatinum), 사이톡산(Cytoxan), 다우노루비신(daunorubicin), DTIC, 5-FU, 플루다라빈(Fludarabine), 하이드리아(Hydra), 아이다루비신(Idarubicin), 이포스파마이(Ifosfamide), 메토크렉사트(methotrexate), 미트라마이신(Mithramycin), 미토마이신(Mitomycin), 미톡산트론(Mitoxantrone), 질소 머스타드, 탁솔(또는 도세탁셀(docetaxel)과 같은 다른 탁산), 벨반(Velban), 빈크리스틴(Vincristine), VP-16을 포함하는 반면에, 일부 이상의 새로운 약물은 겜시타빈(Gemcitabine)(Gemzar), 허셉틴(Herceptin), 이리노테칸(Irinotecan) (캠토소르(Camptosar), CPT-11), 류스타틴(Leustatin), 나벨빈(Navelbine), 리툭산 STI-571(Rituxan STI-571), 탁소텔(Taxotere), 토폠테칸(Topotecan)(하이캄틴(Hycamtin)), 젤로다(Xeloda)(Capecitabine), 제벨린(Zevelin) 및 칼시트리올(calcitriol)을 포함한다.
- [0259] 사용될 수 있는 면역조절제의 비제한적인 예들은 AS-101 (Wyeth-Ayerst Labs.), 브로피리민(bropirimine)(Upjohn), 감마 인터페론(gamma interferon)(Genentech), GM-CSF(과립구큰포식세포집락자극인자(granulocyte macrophage colony stimulating factor); Genetics Institute), IL-2(Cetus 또는 Hoffman-LaRoche), 인간 면역 글로불린(Cutter Biological), IMREG(New Orleans, La.의 Imreg로부터), SK&F 106528, 및 TNF(종양 괴사 인자; Genentech)을 포함한다.
- [0260] 암의 일부 유형을 위한 다른 통상적인 처치는 외과 치료, 예를 들면, 암 또는 이것의 일부의 외과적 절제이다. 다른 치료방법은 방사선 치료로서, 예를 들면 종양을 제거하거나 외과 절제하기 전에 이것을 줄어든게하는 것을 돕기 위해 종양 부위에 방사선 물질 또는 에너지(외부의 빔 치료와 같은)의 투여이다.
- [0261] CDK(사이클린-의존성 키나아제) 저해제는 CDKs의 기능을 억제하는 제제이다. 상기 제공된 방법으로 사용을 위한 CDK 저해제의 비제한적인 예들은 AG-024322, AT7519, AZD5438, 플라보피리돌(flavopiridol), 인디술람

(indisulam), P1446A-05, PD-0332991, 및 P276-00 (예를 들어, Lapenna *et al.*, *Nature Reviews*, 8:547-566, 2009을 참조)을 포함한다. 다른 CDK 저해제는 LY2835219, 팔보시시브(Palbociclib), LEE011 (Novartis), 팬-CDK 저해제 AT7519, 셀리시시리브(seliciclib), CYC065, 부티로락톤 I(butyrolactone I), 히메니알디신(hymenialdisine), SU9516, CINK4, PD0183812 또는 파스카플리신(fascaplysin)을 포함한다.

[0262] 일부 실시예에서, CDK 저해제는 광범위(broad range)의 저해제(플라보피리돌(flavopiridol), 올로모우신(olomoucine), 로스코비틴(roscovitine), 켄파울론(kenpaulone), SNS-032, AT7519, AG-024322, (S)-로스코비틴 또는 R547와 같은)이다. 다른 실시예에서, CDK 저해제는 특이적 저해제(파스카플리신, 류비딘(ryuvidine), 푸르발라놀 A(purvalanol A), NU2058, Bml-259, SU 9516, PD0332991 또는 P-276-00와 같은)이다.

[0263] 제제 및 복용량의 선택은 치료할 질병에 기초하여 당업자에 의해 쉽게 결정될 수 있다. 제제 또는 조성물의 조합은 부수적으로(예를 들어, 혼합물로서), 별도로 그러나 동시에(예를 들어, 별도의 정맥 내 주사라인을 경유하여)투여하거나 또는 순차적으로(예를 들어, 하나의 제제가 첫번째로 투여되고 뒤이어 두 번째 제제가 투여된다) 투여될 수 있다. 따라서, 조합의 용어는 2개 이상의 제제 또는 조성물의 부수적, 동시 또는 순차적 투여를 의미하는 것으로 사용된다.

[0264] 본 명세서에서 개시된 방법에 따르면, 본 명세서에서 제공된 하나 이상의 제제의 유효량이 개체에게 투여된다. 상기 유효량은 원하는 생리학적 반응(예를 들어, 암세포의 살상)을 생성하는데 필요한 양으로 정의한다. 치료제는 일반적으로 매일 약 0.001 mg/kg 내지 약 1000 mg/kg의 초기 복용량으로 투여된다. 약 0.01 mg/kg 내지 약 500 mg/kg, 또는 약 0.1 mg/kg 내지 약 200 mg/kg, 또는 약 1 mg/kg 내지 약 100 mg/kg, 또는 약 10 mg/kg 내지 약 50 mg/kg의 용량 범위가 사용될 수 있다. 그러나, 복용량은 개체의 요구되는 정도, 치료하고자 하는 질환의 심각성, 및 사용되는 화합물에 의존하여 다양할 수 있다. 예를 들면, 복용량은 특정한 개체에서 진단된 암의 유형 및 단계를 고려하여 경험적으로 결정될 수 있다. 제공된 방법의 맥락에서, 개체에 투여되는 용량은 시간이 지남에 따라 환자에게 유익한 치료적 반응을 일으키기에 충분해야만 한다. 특정 상황을 위한 적절한 복용량의 결정은 종사자의 기술에 달렸다. 따라서, 제제를 투여하기 위한 유효량 및 계획은 당업자에 의해 경험적으로 결정될 수 있다. 복용량은 원하지 않는 교차-반응, 과민 반응 등과 같은 실질적 부작용을 야기할 정도로 많으면 안된다. 복용량은 임의의 금기(contraindications) 상황에서 주치의에 의해 조정될 수 있다. 약학적 제제의 종류에 대한 적절한 복용량에 대한 지침을 문헌에서 찾을 수 있다.

[0265] 개시된 바와 같이, 종양세포를 재조합 아데노바이러스, 또는 이의 조성물과 접촉시켜 종양세포 생존능을 저해하는 방법을 여기에서 제공한다. 일부 실시예에서, 방법은 시험관 내 방법이다. 다른 실시예에서, 그 방법은 생체 내 방법이고 종양세포를 접촉시키는 것은 종양이 있는 개체에게 재조합 아데노바이러스 또는 조성물을 투여하는 것을 포함한다.

[0266] 본 명세서에서 개시된 재조합 아데노바이러스(또는 이의 조성물)의 치료학적 유효량을 개체에게 투여하여, 개체의 종양 진행을 저해시키거나 종양 부피를 감소시키는 방법이 또한 제공된다.

[0267] 또한 본 명세서에서 개시된 재조합 아데노바이러스(또는 이의 조성물)의 치료학적 유효량을 개체에게 투여하여, 개체의 암을 치료하는 방법이 제공된다.

[0268] 추가적으로 (i) 라파마이신/라파로그(rapamycin/rapalog) 유도성의 재표적화된(retargeted) 재조합 아데노바이러스의 치료학적 유효량; 및 (ii) 라파마이신 또는 이의 유사체를 개체에게 투여하여 개체의 종양 부피를 감소시키는 방법을 제공한다. 또한 (i) 라파마이신/라파로그(rapamycin/rapalog) 유도성의 재표적화된 재조합 아데노바이러스의 치료학적 유효량; 및 (ii) 라파마이신 또는 이의 유사체를 개체에게 투여하여 개체의 암을 치료하는 방법이 제공된다.

[0269] 일부 실시예에서, 라파마이신 또는 라파마이신 유사체는 약 0.1 mg/kg 내지 약 2.0 mg/kg의 용량으로 투여된다. 다른 실시예에서, 라파마이신은 약 0.1 mg/kg 내지 약 0.5 mg/kg의 용량으로 투여된다. 일부 실시예에서, 라파마이신은 약 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg 또는 2.0 mg/kg의 용량으로 투여된다.

[0270] 일부 실시예에서, 라파마이신 또는 라파마이신 유사체는 약 3 내지 12 mg/m², 또는 약 5 내지 10 mg/m²와 같은, 약 1 내지 15 mg/m²의 용량으로 투여된다. 일부 실시예에서, 라파마이신 또는 라파마이신 유사체는 약 1, 약 2, 약 3, 약 4, 약 5, 약 6, 약 7, 약 8, 약 9, 약 10, 약 11, 약 12, 약 13, 약 14 또는 약 15 mg/m²의 용량으로 투여된다.

[0271] 일부 실시예에서, 라파마이신 또는 라파마이신 유사체는 개체에 실질적 면역억제의 원인을 일으키지 않는 용량

으로 부여된다. '실질적 면역억제의 원인을 일으키지 않는 용량'의 라파마이신 또는 이의 유사체의 투여는 특정한 개체에 대하여 유효한 농도 EC_{25} 보다 적거나 동등한 용량을 의미한다. 당업자는 개체에서 실질적 면역억제를 일으키지 않는 라파마이신 또는 이의 유사체의 용량을 결정할 수 있다(예를 들어, Schubert *et al.*, *Am J Transplant* 4:767-773, 2004를 참조). 일부 실시예에서, EC_{25} 는 약 3 ng/ml 내지 약 10 ng/ml와 같은, 약 1 ng/ml 내지 약 15 ng/ml이다. 비제한적인 실시예에서, EC_{25} 는 약 1 ng/ml, 약 2 ng/ml, 약 3 ng/ml, 약 4 ng/ml, 약 5 ng/ml, 약 6 ng/ml, 약 7 ng/ml, 약 8 ng/ml, 약 9 ng/ml, 약 10 ng/ml, 약 11 ng/ml, 약 12 ng/ml, 약 13 ng/ml, 약 14 ng/ml 또는 약 15 ng/ml이다.

[0272] 일부 실시예에서, 재표적화된(retargeted) 아데노바이러스의 게놈은 FKBP에 융합된 표적 리간드, 및 야생형 FKBP-라파마이신 결합(FRB) 단백질 또는 T2098L 치환을 포함하는 돌연변이 FRB 단백질에 융합된 아데노바이러스 섬유 단백질을 암호화한다. 일부 실시예에서, 표적 리간드는 EGFR 특이적인 단일 도메인 항체와 같은, 단일 도메인 항체이다. 특정 비제한적인 예에서, 재조합 아데노바이러스의 게놈은 AdSyn-C0335(서열번호 30), AdSyn-C0335B-K(서열번호 92) 또는 AdSyn-C0335B-TK(서열번호 97)의 뉴클레오타이드 서열을 포함한다.

[0273] 방법의 일부 실시예에서, 종양 또는 암은 E2F 경로의 비정상적인 조절(deregulation)이 특징이다. 방법의 일부 실시예에서, 종양 또는 암은 폐, 전립선, 직장, 유방, 갑상선, 신장 또는 간의 종양 또는 암이거나, 또는 백혈병이다

[0274] 일부 실시예에서, 방법은 화학치료제, 면역 조절제 또는 CDK 저해제, 예를 들면 CDK4 저해제와 같은 추가적인 치료제로 개체를 치료하는 것을 더 포함한다.

[0275]

[0276] 하기의 예는 특정한 특성 및/또는 실시예를 설명하는 것을 제공한다. 하기 실시예가 설명된 특정한 특성 또는 실시예에 대한 개시를 제한하는 것으로 해석되어서는 안된다.

[0277] **실시예**

[0278] **실시예 1: 비정상적인 E2F 활성을 가진 종양 세포에서 선별적으로 복제하는 종양 살상 아데노바이러스**

[0279] 변형된 아데노바이러스는 본 명세서에서 참고로 인용된, PCT 공개번호 WO 2012/024351에서 설명된 방법에 따라, 하기의 참조된 구성요소로 만들었다. 게이트웨이 pDONR 벡터를 사용하였다. 인간 Ad5 DNA로부터, E1 모듈을 PCR로 얻고 SLIC를 사용하여 벡터 pDONR P1P4로 삽입하였다. attL1 및 attL4 재조합 부위를 포함하는 pDONR P1P4 벡터 백본(backbone)을 PCR을 이용하여 증폭하였고 SLIC로 Ad5 E1 모듈과 결합시켰다. E3 모듈을 PCR하여 attB5 및 attB3r 재조합 부위가 양 측면에 위치한(flanked) 생성물을 생성하였다. 생성물을 게이트웨이 BP 반응에 의해 pDONR P5P3r 벡터로 삽입하였다. E4 모듈을 PCR하여 attB3 및 attB2 재조합 부위가 양측면에 위치한 생성물을 얻었다. 생성물을 게이트웨이 BP 반응으로 pDONR P3P2 벡터로 삽입하였다. pDONR P5P2 벡터로부터 attR5-ccdB-Cm(r)-attR2 단편을 PCR로 증폭시키고 에드셈블리 데스트 벡터(Adsembly DEST vector)로 삽입하였다(“MultiSite Gateway®ProPlus”, Cat# 12537-100; 및 Sone *et al.*, *J Biotechnol* 136(3-4):113-121, 2008를 참조). 에드셈블리(Adsembly) 방법은 전부 본 명세서에 참조문헌으로 삽입된, PCT 공개번호 WO 2012/024351에 기술되어 있다.

[0280] 에드셈블리 데스트 벡터(Adsembly DEST vector)를 위한 벡터 백본은 3개의 다른 출처 유래의 부분들로 구성된다. Amp(r) 카세트 및 lacZ 유전자를 플라스미드 pUC19로부터 증폭하였다. 이것을 BioBricksiGEM 2007 부품(parts) 배포의 일부이며, 플라스미드 pSB3K5-I52002로부터 얻은, p15A 복제원점(p15A origin의 replication)과 결합시켰다. 낮은 복제 수(10-12)의 플라스미드를 유지하는 p15A ori는 E1 독성을 감소시키는데 필요하다. 마지막으로, 자가-절단 바이러스(self-excising virus)를 만들기 위해서, 효소 ISceI에 대한 포유동물 발현 카세트를 플라스미드 pAdZ5-CV5-E3+로부터 PCR 증폭시켰다. 상기 카세트를 벡터 백본으로 클로닝하여 p15A-SceI라고 불리는 벡터로 제조하였다. 이것은 게놈 조립(assembly)을 시작하기 위해 사용될 벡터이다. 유전자 모듈을 야생형 Ad5 바이러스로부터 정제된 DNA 또는 플라스미드 pAd/CMV/V5/DEST(인비트로젠)로부터 얻었다.

[0281] 인간 Ad5에 대한 DEST 벡터에 관해서, E2 및 L3 모듈을 3-단편 SLIC에 의해 플라스미드 p15A-SceI로 삽입하였다. attR5 및 attR2 부위가 양측면에 위치한 ccdB 및 Chlor(r)를 발현하는 역선택 마커를 플라스미드 pDONR P5P2로부터 PCR하여 얻었다. 두번째 역선택 마커를 벡터 pDONR P1P4로부터 PCR하여 얻었다. 2개의 역선택 마커를 E2 및 L4 모듈 끝에 조작된 고유의 제한효소로 자른 후에 SLIC로 p15A-SceI E2-L4의 오른쪽 및 왼쪽에 삽입하여 DEST 벡터를 만들었다.

- [0282] Amp(r) 카세트: 플라스미드 pUC19에 관하여, p15A ori: 플라스미드 pSB3K5-I52002는 BioBricksiGEM 2007 부품(parts) 배포의 일부이다. 아데노바이러스 유전자 모듈에 관하여, DNA를 Ad5 입자 또는 플라스미드 pAd/CMV/V5/DEST(인비트로젠)으로부터 정제하였다. DONR 벡터 pDONR P1P4, P5P2, P5P3R, P3P2를 인비트로젠으로부터 입수하였다.
- [0283] 모든 PCR 분석은 퓨전(Phusion) 효소(NEB)를 사용하여 수행하였다. Ad5로부터 아데노바이러스 유전자(ADENOVIRAL GENE) 모듈을 얻기 위한 PCR 분석은 1x HF 버퍼, 200 μ M의 각각의 dNTP, 0.5 μ M의 각각의 프라이머, 및 10 ng의 템플레이트로 수행되었다. E2-L2 모듈을 위하여, 3% DMSO를 첨가하였다. 템플레이트는 플라스미드 pAd/PL-DEST(인비트로젠; E2-L2, L3-L4, 및 E4 모듈을 위한)이거나 또는 Ad5 게놈 DNA(E1 및 E3 모듈을 위한)이었다. PCR 조건은 하기와 같다:
- [0284] E2-L2 및 L3-L4: 98°C 30초 - 98°C 10초, 65°C 30초 (매 2 사이클마다 온도 1°C 감소), 72°C 7분의 10 사이클 - 98°C 10초, 60°C 30초, 72°C 8분의 29 사이클 - 72°C 10분 - 4°C 유지.
- [0285] E3: 98°C 30초 - 98°C 10초, 70°C 30초 (매 사이클마다 온도 0.5°C 감소), 72°C 2분30초의 10 사이클 - 98°C 10초, 68°C 30초, 72°C 2분30초의 25 사이클 - 72°C 10분 - 4°C 유지.
- [0286] E4: 98°C 30초 - 98°C 10초, 63°C 30초 (매 사이클마다 온도 0.5°C 감소), 72°C 2분의 6 사이클 - 98°C 10초, 60°C 30초, 72°C 2분의 29 사이클 - 72°C 5분 - 4°C 유지.
- [0287] 정제된 바이러스로부터 바이러스 게놈 DNA를 얻기 위하여, 정제된 바이러스의 100 μ l를 10 mM 트리스 pH8, 5 mM EDTA, 200 mM NaCl, 및 0.2% SDS를 포함하는 300 μ l의 용해 버퍼에 첨가했다. 혼합물을 60°C에서 5분 동안 배양하고, 이어서 5 μ l의 프로테이나제 K(~20 mg/ml)를 추가하고 1 시간 동안 60°C에서 추가로 배양하였다. 그런 다음 시료를 5분 동안 얼음에 방치하고, 이어서 15분 동안 15K x g에서 회전(spinning)시켰다. 상층액을 제거하고 동일 부피의 이소프로판올을 첨가하고, 잘 혼합하여 4°C에서 15분 동안 15K x g에서 회전하였다. 펠렛을 70% 에탄올로 세척하였고 4°C에서 15분 동안 재회전시켰다. 펠렛을 건조시키고 재현탁하여 사용하였다.
- [0288] SLIC에 관하여, 선형 단편(linear fragment)을 하기의 20 μ l 반응에서 상온에서 20분 동안 엑소뉴클리즈(exonuclease)로 처리되었다: 50 mM 트리스 pH8, 10 mM MgCl₂, 50 μ g/ml BSA, 200 mM 우레아, 5 mM DTT, 및 0.5 μ l T4 DNA 폴리머라제. 반응을 1 μ l 0.5M EDTA를 첨가하여 중지시키고, 이어서 20분 동안 75°C에서 배양하였다. 동일한 양의 T4-처리된 DNA를 새로운 튜브의 대략 20 μ l 부피로 혼합하였다. 2개의 단편을 혼합한 SLIC에 대하여, 각각의 반응액의 10 μ l를 사용하였다. 3개의 단편을 혼합한 SLIC에 대하여, 각각의 반응액의 7 μ l를 사용하였다. 단편을 10분 동안 65°C로 가열하여 어닐링(annealed)하였고, 이어서 매 5 초마다 온도를 0.5°C 천천히 감소시켜 25°C로 낮추었다. 어닐링한 후, 반응액의 5 μ l를 형질전환시키고 클론을 스크리닝하였다.
- [0289] AdSLIC에 관하여, 3-단편 SLIC 반응을 100 ng의 T4-처리된 p15A-SceI (PCR에 의해 직선화됨), 및 각각의 E2 및 L3 모듈의 300 ng을 사용하여 수행하였다(이들의 각각의 엔트리 벡터로부터 PCR에 의해 수득하였다). 벡터 p15A-SceI E2-L4이 만들어졌다. 5 μ g의 p15A-SceI E2-L4를 SwaI로 자르고 Qiagen QiaexII을 사용하여 젤 정제화하였다. E3 및 E4 모듈을 이들의 각각의 엔트리 벡터로부터 PCR에 의해 수득하였다. 각각의 직선화된 벡터(450 ng) 및 PCR 생성물(200 ng)을 T4 DNA 폴리머라제로 처리하고 SLIC를 150-200 ng의 벡터 및 ~100 ng의 각각의 모듈 PCR을 사용하여, 통상적인 방법으로 수행하였다. 양성 클론을 분리한 후, 5 μ g의 새로운 벡터를 PacI로 자르고 젤 정제한 다음, 새로운 SLIC 반응에서 E1 PCR 생성물(100 ng의 T4-처리된)과 결합시켰다. 게놈 조합이 완성되었고, 바이러스를 재구성하기 위한 형질감염을 위한 플라스미드가 준비되었다.
- [0290] E1 및 E4 돌연변이의 영역을 제조하기 위하여, 각각의 모듈 엔트리 벡터 상에서 조작을 수행하였다. 벡터 백본이 있는 E1 모듈을 LTCHE 서열(서열번호 32의 122-126잔기)이 결합된 생성물을 생성하기 위한 프라이머로 PCR 증폭한 후, SLIC을 사용하여 원형으로 하여(circularized) pENTR-E1-E1A- Δ LXCXE를 생성하였다.
- [0291] 또한, pENTR-E1-E1A-Y47H, pENTR-E1-E1A-C124G, 또는 pENTR-E1-E1A- Δ 2-11을 각각 생성하기 위하여 벡터 백본을 가진 E1 모듈을 Y47을 H로, C124를 G로 변이시키거나 또는 잔기 2-11을 제거하기 위한 E1A 코돈 변이들(ENTR-E1-E1A-Y47H-C124G 및 pENTR-E1-E1A-Y47H-C124G- Δ 2-11)을 가진 생성물을 생성하기 위하여 프라이머로 PCR 증폭하였다. 상기 생성물을 상기 돌연변이 조합을 생성하기 위한 추가 PCR 돌연변이를 위하여 템플레이트로 사용하였다. 벡터 백본을 가진 E4 모듈을 E4orf6 종결코돈의 하류(downstream)에 E4orf6/7-특이적 엑손 서열(297 bp)이 결합된 생성물을 생성하기 위한 프라이머로 PCR 증폭하여 pENTR-E4- Δ E4orf6/7을 생성하였다. 상기 생성물은 E4orf1의 PDZ-결합 모티프, 또는 전체 E4orf1 서열이 결합된(각각 pENTR-E4- Δ E4orf6/7-E4orf1 Δ PDZb 및 pENTR-E4- Δ E4orf6/7- Δ E4orf1) 생성물을 생성하기 위한 프라이머로 PCR을 하기 위한 템플레이트로서 또한

사용될 수 있다.

[0292] 돌연변이를 가진 완전한 바이러스 게놈을 생성하기 위하여, 야생형 E3 모듈 및 야생형 E4 또는 돌연변이의 E4 모듈, 그런 다음 야생형 E1 또는 돌연변이의 E1과 조합한 p15A-SceI E2-L4를 사용하여 AdSlicR이 수행되었다. 야생형 AdSlicR 아데노바이러스를 하기의 표 1에서 표기하였다.

[0293] 표 1. E1 및 E4에서 변형을 가진 아데노바이러스

표 1

[0294]

바이러스 명칭	E1	E4	서열번호
AdSyn-C0102	wt	wt	1
AdSyn-C0210	wt	ΔE4orf6/7	2
AdSyn-C0283	wt	E4orf1 ΔPDZb, ΔE4orf6/7	3
AdSyn-C0284	wt	ΔE4orf1, ΔE4orf6/7	4
AdSyn-C0189	E1A ΔLXCXE	wt	5
AdSyn-C0181	E1A ΔLXCXE	ΔE4orf6/7	6
AdSyn-C0285	E1A ΔLXCXE	E4orf1 ΔPDZb, ΔE4orf6/7	7
AdSyn-C0286	E1A ΔLXCXE	ΔE4orf1, ΔE4orf6/7	8
AdSyn-C0235	E1A C124G	wt	9
AdSyn-C0287	E1A C124G	ΔE4orf6/7	10
AdSyn-C0288	E1A C124G	E4orf1 ΔPDZb, ΔE4orf6/7	11
AdSyn-C0289	E1A C124G	ΔE4orf1, ΔE4orf6/7	12
AdSyn-C0236	E1A d2-11	wt	13
AdSyn-C0290	E1A d2-11	ΔE4orf6/7	14
AdSyn-C0291	E1A d2-11	E4orf1 ΔPDZb, ΔE4orf6/7	15
AdSyn-C0292	E1A d2-11	ΔE4orf1, ΔE4orf6/7	16
AdSyn-C0238	E1A Y47H, C124G	wt	17
AdSyn-C0293	E1A Y47H, C124G	ΔE4orf6/7	18
AdSyn-C0294	E1A Y47H, C124G	E4orf1 ΔPDZb, ΔE4orf6/7	19
AdSyn-C0295	E1A Y47H, C124G	ΔE4orf1, ΔE4orf6/7	20
AdSyn-C0244	E1A Y47H, C124G, d2-11	wt	21
AdSyn-C0296	E1A Y47H, C124G, d2-11	ΔE4orf6/7	22
AdSyn-C0297	E1A Y47H, C124G, d2-11	E4orf1 ΔPDZb, ΔE4orf6/7	23
AdSyn-C0298	E1A Y47H, C124G, d2-11	ΔE4orf1, ΔE4orf6/7	24

[0295] 바이러스 생성물, 농도 및 정제에 관하여, 293 E4 세포를 감염 입자로 감염시키고, CPE가 분명해지는 감염 후 약 48시간 쯤에, 세포를 수득하고 5분 동안 500 x g 에서 원심분리를 통해 분리하였다. 세포를 TMN 버퍼(10 mM TrisCl pH 7.5, 1 mM MgCl₂, 150 mM NaCl)상에서 냉동/해동을 3번 반복하여 용해하였고, 세포 잔해는 15분 동안 3,000 x g 및 3,500 x g 에서 2회 원심분리하여 제거되었다. 세슘 클로라이드 구배(cesium chloride gradient)(0.5 g/ml)는 18 내지 24시간 동안 37,000 x g에서 초고속원심분리를 통한 바이러스 입자의 밴드(band)화에 이용되었다. 밴드를 수득하고 4℃에서 하룻밤(12-18시간) 동안 10% 글리세롤을 포함한 TMN에 있는 10,000 MWCO Slide-A-Lyzer® 투석 카세트(Thermo Scientific) 상에서 투석하였고, -80℃에서 저장하였다. 정제된 바이러스의 역가(titer)는 항-아데노바이러스 유형 5개의 주요 항체(ab6982, Abcam), 및 ImmunoPure 항-래빗 알칼린 포스파타제 2차 항체(Thermo Scientific)를 이용, 세포-기반 순차적 희석(cell-based serial dilution) 감염 ELISA로 역가가 결정된 야생형 균주와 비교하여 결정되었다.

[0296] 1차 인간 소기도 상피세포(SAEC)의 감염 동안에 아데노바이러스 단백질 발현을 평가하기 위하여, 2-웰 플레이트에서 휴지기상태의 SAEC를 MOI 30 아데노바이러스로 감염시키고, 감염후 4 시간째에 배지를 교체하였다. 감염 후 24, 36, 및 48시간째에, 세포를 차가운 PBS로 세척하였고, 500 μl의 차가운 PBS에서 수득, 4℃에서 5분 동안 5,000 rpm에서 펠렛화하고, 재빨리 냉각하여 -80℃에서 저장하였다. 세포 펠렛을 컵 초음파파쇄기(cup sonicator) (4℃에서 60 앰플리튜드 2x 60 초 펄스)로 초음파처리(sonocation)를 포함하여, RIPA 버퍼(100 mM 트리스 pH7.4, 300 mM NaCl, 2mM EDTA, 2% 트리톤 X, 2% 데옥시콜레이트, 2 mM NaF, 0.2 mM NaVO₄, 2 mM DTT)에서 4℃에서 1 시간 동안 용해시켰다. 세포 잔해를 4℃에서 20분 동안 13,000 rpm에서 원심분리하여 펠렛화시켰다. 단백질 농도는 Bio-Rad의 DC™ 단백질 분석을 사용하여 결정되었고, 시료의 단백질 농도는 정규화

(normalization)되었다. Life Technologies Novex® NuPAGE® SDS-PAGE 젤을 사용하여, 제조사의 프로토콜에 따라, 젤 전기영동을 수행하였다. 단백질을 웨스턴 블랏으로 검출하였다. 단백질 검출에 사용된 1차 항체는 다음과 같다: E1A(ab28305, Abcam), β -액틴(A5441, Sigma), Ad5 후기(late) 단백질(ab6982, Abcam), 사이클린 A(Ab-6 6E6, NeoMarkers), 사이클린 B(Ab-3 GNS1, NeoMarkers). 알렉사 플루오로® 항체(Life Technologies)를 2차 항체로서 사용하였고, 신호를 LI-COR ODYSSEY® 기계를 사용하여 검출하였다. 폐 선암 A549 세포 및 정상적인 인간 정상세포 (NHA)를 감염시키는 동안에 아데노바이러스 단백질 발현을 측정하기 위하여, 12-웰 플레이트 상의 가득찬 세포를 MOI 10의 아데노바이러스로 감염시키고, SAEC에 대해서 설명된 바와 같이 유사하게 처리하였다. 교모세포종 U87 세포, 교모세포종 U118 세포, 인간 혈관 내피세포(HuVEC), 또는 인간 섬유아세포의 감염 동안에 아데노바이러스 단백질 발현을 측정하기 위하여, 12-웰 플레이트 상의 가득찬 세포를 MOI 20의 아데노바이러스로 감염시키고, SAEC에 대해서 설명된 바와 같이 유사하게 처리하였다.

[0297] 세포주기 분석을 수행하기 위하여, 단백질 발현에 수행한 바와 같이 세포를 동일한 MOI로 감염시켰다. 감염 후 48시간째에, 세포를 플레이트에서 트립신화하고 차가운 PBS로 세척하였다. 세포를 500 μ l 차가운 PBS에 재현탁하고, 3 ml의 차가운 70% EtOH/15 mM 글라이신, pH 2.8 용액에서 고정하였다. 시료를 4°C에 보존하고, FACS 분석에 앞서 세포를 펠렛화하고, 차가운 PBS로 세척하고, 프로피디움 요오드화물 (PI)/RNase A 용액에서 재현탁한 후, 1시간 동안 37°C에서 배양하였다. 각각 시료에 대하여 FACS에 의해 적어도 10,000 이벤트(event)를 수득하였다.

[0298] 감염으로부터 아데노바이러스 버스트(burst)를 평가하기 위하여, 12-웰 플레이트의 휴지기상태의 세포를 감염다중도 1 또는 감염다중도 10의 아데노바이러스로 감염시키고, 감염 후 4시간째에 배지를 세포상에서 교체하였다. 웰로부터의 배지를 감염 후 48 및 72시간째에 수득하였고, 재빨리 냉동시키고 1회 해동시킨 후, 세포 잔해를 펠렛화시키기 위해 5분 동안 4°C에서 7,000 rpm에서 원심분리하였다. 배지의 부피를 측정하고 재빨리 냉동시켜 -80°C에서 저장하였다. 배지의 역가(titer)는 항-아데노바이러스 타입 5 1차 항체 (ab6982, Abcam) 및 ImmunoPure 항-래빗 알칼린 포스파타제 2차 항체(Thermo Scientific)를 이용, 세포-기반 순차적 회색 감염 ELISA로 역가가 결정된 야생형 균주와 비교하여 결정되었다.

[0299] 세포 생존능 분석에 관하여, 세포를 96-웰 플레이트에 분주하였고, MOI 30에서 연속 희석으로 트리플리케이트(triplicate) 감염시켰다. 감염 후 7-10 일에 MOI 10으로 감염된 웰에서 완전한 CPE가 형성되었을 때, 대사 활성을 세포 증식제 WST-1 (Roche)를 사용하여 제조사의 프로토콜에 따라 측정하였다.

[0300] 상기에서 논의한 바와 같이, 암은 추가적 치료제를 필요로 하는 골칫거리 질병이다. 치료제의 하나는 종양 살상 바이러스를 포함한다. 아데노바이러스는 종양 살상 바이러스로서 사용을 위해 연구되고 있는 바이러스의 하나이다. 망막아세포종(Rb) 종양 억제인자 경로(Rb tumor suppressor pathway) 기능은 Rb의 직접적 돌연변이에 의해, CDK-저해제인 p16 기능(예를 들어 돌연변이/메틸레이션에 의해)에 의해, 또는 CDK/사이클린의 증폭에 의해 거의 모든 인간 암에서 소실되었다. 정상적 비-분열 세포에서, Rb는 저인산화 상태(hypophosphorylated)를 유지되고, 자체의 표적 프로모터에서 전사인자 E2F에 결합, 크로마틴-재형성 복합체(chromatin-remodeling complexes) 및 히스톤-변형 활성 도메인(histone-modifying activities domain)을 리쿠르트(recruit)할뿐만 아니라 E2F 전사촉진 도메인을 차폐(masking)하여 전사를 억제한다. 세포주기의 G1-S 전이 동안에, CDKs는 E2F 억제를 완화하는 Rb를 인산화시킨다. 아데노바이러스는 세포를 바이러스 계놈을 복제하고 재생산하도록 만들기 위해 Rb 종양 억제인자 경로를 불활성화시키는 초기 바이러스 종양 단백질(viral oncoprotein)을 발현한다. 아데노바이러스 E1A는 부분적으로, LXCXE 모티프를 경유하여, Rb의 종양 억제인자 활성을 비정상적으로 조절하면서 Rb에 결합한다. 따라서 E1A에 LXCXE 모티프를 삭제하는 것은 Rb의 불활성화를 제거할 것이고, 선별적으로 복제하는 바이러스 (ONYX-838)를 만들 것으로 생각된다. 그러나, Johnson *et al.* (Cancer Cell, 1(4):325-337, 2002)은 본 명세서에 기술된 실험결과와 상응하게, Ad E1A LXCXE 돌연변이가 S-단계 진입, 바이러스 DNA 복제, 및 후기(late) 단백질 발현을 방지하는데 충분하지 않다는 증거를 제공한다. E1A 돌연변이의 Rb-선택성(selectivity)은 논쟁이 있음에도 불구하고, 상기 돌연변이는 임상 시험 2상(phase II clinical trials)으로 들어가고 있는 악성 뇌종양에 대한 종양 살상 바이러스(DNX-2401)의 기초로서 추진되고 있다. 높은 Rb-선택성(selectivity)을 얻기 위한 시도에서, 아데노바이러스의 E1 및 E4 영역(region)에 대한 프로모터를 E2F의 프로모터로 치환하고, ONYX-411을 생성하기 위하여 E1A Δ LXCXE 모티프와 조합된 아데노바이러스를 생성하였다 (Johnson *et al.*, Cancer Cell, 1(4):325-337, 2002; 및 Dubensky *et al.*, Cancer Cell, 1(4):307-309, 2002). 상기 바이러스를 테스트하기 위하여, 종양 및 초대 인간 세포를 야생형 바이러스, ONYX-838 (E1A Δ LXCXE) 또는 ONYX-411로 감염시켰고 감염 후에 다양한 시점에서 수득하였다. ONYX-838은 종양 및 주요 폐 상피 세포에서 분별없이 복제한다. 아데노바이러스 E1A, E1B 및 E4 영역의 세포내 E2F에 의한 조절과 조합된 E1A Δ

LXCXE를 가진 ONYX-411는 정상 세포와 대비하여 종양 세포에서 선별적-복제를 나타냈다(Johnson *et al.*, *Cancer Cell* 1(4):325-337, 2002). 그러나, 종양세포에서 E2F 프로모터는 재조합이 일어나 야생형 바이러스 수준으로 복제가 제한되었다.

- [0301] 본 명세서에서 설명된 바와 같이, E1A의 Rb 억제를 통한 E2F의 방출(release)과는 독립적으로, 세포 내 프로모터 및 Ad 프로모터에서 E2F 단백질을 추가로 안정화시키는 다른 Ad 단백질, E4orf6/7이 있다. E1A 및 E4orf6/7은 함께 E2F에 의한 전사를 유도, S-단계 개시 및 그에 따른 바이러스 DNA 게놈의 증식을 일으킨다. 따라서, 기능적 Rb가 결여된 종양 세포에 선택적인 종양 살상 바이러스 치료제인 E1A 변형, E4orf1 변형 및/또는 E4orf6/7 변형을 가지는 재조합 아데노바이러스가 여기에 제공된다. 특정적으로, E1A/E4orf1/E4orf6/7에서 화합물 돌연변이는 초대 세포가 아닌 종양 세포에서 선별적으로 복제될지를 결정하도록 조작되었다. 상기 돌연변이의 조합은 암에 있어서 효과적이고, 자기-증폭적 치료제가 될 수 있음이 제안된다.
- [0302] 정상(non-tumor) 세포 및 종양 세포에서 상기 바이러스를 테스트하기 위하여, 세포를 mock 감염하거나 또는 AdSyn-C0102(야생형), AdSyn-C0181(E1A ΔLXCXE/ΔE4orf6/7), AdSyn-C0189 (E1A ΔLXCXE), 또는 ONYX-838 (E1A ΔCR2)으로 감염시켰다. 또한 ONYX-838은 E1A의 CR2 도메인에 있는 ΔLXCXE가 없다. 휴지기상태의 인간 초대 소기도 상피세포(SAEC)(비종양 세포)는 감염다중도 10으로 감염되었다. AdSyn-C0102는 감염의 후기 시기(later times)에 E1A 수준의 예상된 바와 같이 감소를 나타냈다(도 1A). 유사하게, AdSyn-C0189 또는 ONYX-838로 감염된 세포에서, 감염의 후기 시기에 E1A 발현 수준의 감소가 있다; 그러나, 보다 초기에는, E1A의 발현이 AdSyn-C0102가 감염된 세포에서의 E1A의 발현보다 높았다. 반대로, AdSyn-C0181은 감염 전반에 걸쳐 강하고 지속된 E1A 발현을 나타냈으며, 이것은 아데노바이러스 생활사(lifecycle)를 통한 진행이 실패했음을 나타낸다. 가득찬(confluent) 폐 선암(A549) 세포(종양세포)는 MOI 30에서 감염시켰다. 모든 감염은 감염 동안의 후기 시기에 E1A 수준의 예측된 감소를 초래하였으며(도 1B), 이는 전형적인 아데노바이러스 생활사 진행을 의미한다.
- [0303] SAEC 또는 A549 세포를 돌연변이 아데노바이러스로 감염시킨 후에 후기(Late) 아데노바이러스 단백질 및 세포내 사이클린 단백질 발현을 도 2A(SAEC) 및 도 2B(A549 세포)에 나타냈다. 야생형 바이러스 및 E1A 돌연변이 단백질을 가진 바이러스와 대조적으로, AdSyn-C0181은 SAEC에 있는 E2F-의존적 세포주기 표적, S 단계, 사이클린 A 및 사이클린 B를 활성화하지 못하였다. 추가적으로, E4orf6/7 돌연변이를 가진 AdSyn-C0181 및 AdSyn-C0201은 SAEC에 있는 후기(Late) 단백질 발현 및 복제가 결함이다. 상기 결함 모두는 AdSyn-C0210에 더 적은 것이 명백하다. 감염된 초대 세포와 대조적으로, A549 세포에서 후기 구조 단백질의 발현에 결함이 없었고, 사이클린 A 및 사이클린 B는 감염된 A549 세포 모두에 존재한다.
- [0304] 감염된 SAEC 및 A549 세포의 DNA 복제는 도 3A 및 3B에 나타났다. AdSyn-C0181 감염은 AdSyn-C0102에 상대적으로 SAEC에서 강한 DNA 복제결함을 나타냈고, 이것은 감소된 바이러스 복제와 연관되어 있다. 사소한(modest) 결함은 이 시점의 AdSyn-C0210 감염된 SAEC에서 또한 뚜렷하다. A549 세포에서 나타나지 않았다.
- [0305] 도 4A-4B는 감염된 SAEC 및 A549 세포로부터 아데노바이러스 버스트(burst)를 나타낸다. AdSyn-C0181 및 AdSyn-C0210 감염 모두는 AdSyn-C0102와 네 비해 SAEC에서 강한 복제 결함을 나타냈다(도 4A). AdSyn-C0210을 제외한, A549 세포에서 바이러스 복제의 결함이 없었다(도 4B).
- [0306] 도 5A-5B는 감염 7일 후에 SAEC 및 A549의 세포 생존능을 나타낸다. AdSyn-C0102와 비교하여, AdSyn-C0181은 SAEC에서 감소된 세포 살상능을 나타낸다. 도 5B에 나타난 바와 같이, 테스트된 돌연변이 바이러스는 A549 세포에서 야생형 바이러스와 비교하여 세포 살상에 결함이 없었다.
- [0307] 또한, 재조합 바이러스는 정상적인 인간 성상세포(NHA)(도 6A-6C), 교모세포종 U87 세포(도 7A-7C), 교모세포종 U87 세포(도 8A-8C) 및 IMR90 세포(도 9A-9C)에서 테스트되었다. NHA에서, AdSyn-C0181은 사이클린 A를 유도하지 않고, AdSyn-C0102와 비교하여 지연되고 감소된 후기 바이러스 단백질 발현을 나타냈으며, 이것은 복제 결함을 표시한다(도 6A). 추가적으로, 감염 입자의 수(도 6B) 및 DNA 함량(도 6C)에 의해 측정된 것처럼, AdSyn-C0181은 야생형 AdSyn-C0102와 비교하여, NHA에서 약화된 감염을 나타냈다. NHA 세포와는 대조적으로, 돌연변이 아데노바이러스는 교모세포종 U87 세포(도 7A-7C) 또는 교모세포종 U118 세포(도 8A-8C)에서 복제결함을 나타내지 않았다. AdSyn-C0181은 IMR90 세포에서 사소한 복제 결함을 나타냈다(도 9A-9C).
- [0308] 따라서, 상기에서 설명된 데이터는 야생형 및 E1AΔCR2 바이러스와는 대조적으로, E1AΔCR2/ΔE4orf6/7 및 ΔE4orf6/7 바이러스는, 캡시드(capsid) 단백질 발현 부족, E2F 표적 유전자(cyclin A 및 B)의 발현 유도 실패, S 단계 진입 및 바이러스 복제의 실패로 증명된 것처럼, 초대 세포에서 복제를 거의 하지 못한다. 반대로, 상기 바이러스는 A549 세포 및 일련의 종양 세포주들에서 야생형 바이러스 수준 만큼 복제한다. 따라서, 본 명세서에

서 개시된 재조합 아데노바이러스는 선별적 암 치료제이다.

[0309] E4orf1에서 돌연변이를 포함하여(표 1 참조), 돌연변이 아데노바이러스들의 복제 특성의 결과를 도 10-15에 나타냈고, 도 16에 요약하였다. 도 10은 감염 10일 후에 초대 정상 인간 성상세포(NHA)의 세포 생존능을 나타내는 그래프이다. 도 11은 감염 9 일후에 휴지기상태의 정상 소기도 상피세포 (SAEC-hTERT)의 세포 생존능을 나타내는 그래프이다. 도 12는 감염 10 일후에 증식하는 SAEC-hTERT 세포의 세포 생존능을 나타내는 그래프이다. 도 13은 감염 7 일후의 인간 폐 선암 세포(A549)의 세포 생존능을 나타내는 그래프이다. 도 14는 감염 7 일후의 인간 유방암 세포(MDA MB 231)의 세포 생존능을 나타내는 그래프이다. 도 15는 감염 7 일후의 감염된 교모세포종 세포 (U87)의 세포 생존능을 나타내는 그래프이다. 도 16은 감염 7 일후의 초대 NHA, SAEC-hTERT(휴지기상태의), SAEC-hTERT(증식), A549, MDA MB 231, 및 U87 세포의 세포 생존능 분석의 정량화 값을 나타낸 히트맵 표 (heatmap table)이다. 추가적으로, 도 17은 감염된 U2OS 및 SaOS2 세포에 대하여 세포 생존능 분석의 정량화 값을 나타낸 표를 제공한다. U2OS 및 SaOS2 세포 모두는 골세포종(osteocarcinoma) 세포이다; 그러나, p53 및 Rb는 U2OS 세포에서 기능적이지만, SaOS2 세포에서 변이되어 있다. 상기 결과는 돌연변이 바이러스가 Rb-결합 종양 세포에서 선별적으로 복제하는 것을 나타낸다.

[0310] 상기 데이터는 E1A, E4orf1 및/또는 E4orf6/7의 다양한 변형의 조합이 Rb 종양 억제인자 경로의 결함을 가진 가진 암세포에서 특이적으로 복제하는 선별적 종양 살상 아데노바이러스를 만들어낼 수 있음을 보여준다.

[0311] 실시예 2: E1, L3, E3 및/또는 E4에서 변형을 가진 종양 살상 아데노바이러스

[0312] 이 실시예는 종양 선별력을 가지는 추가 재조합 아데노바이러스를 기술한다. 전형적 재조합 바이러스는 표 2에 열거되었고 이들 바이러스의 게놈 서열은 서열번호 25-31로 기재되었다.

[0313] 표 2. E1, L3, E3 및/또는 E4에서 변형을 가진 아데노바이러스

표 2

바이러스 명칭 (서열번호)	E1	L3	E3	E4
AdSyn-C0312 (25)	E1A ΔLXCXE	wt	ΔE3-RID α / β , 14.7k +EGFRVHH-FKBP FRB*-섭유	ΔE4orf6/7
AdSyn-C0313 (26)	E1A ΔLXCXE	wt	ΔE3-RID α / β , 14.7k +EGFRVHH-FKBP FRB*-섭유	ΔE4orf6/7
AdSyn-C0199 (27)	ΔE1 +EF1 α -루시퍼라제	wt	wt	wt
AdSyn-C0200 (28)	ΔE1 +EF1 α -루시퍼라제-miR122	wt	wt	wt
AdSyn-C0171 (29)	ΔE1 +EF1 α -루시퍼라제-miR122	핵손 E451Q	wt	wt
AdSyn-C0335 (30)	E1A ΔLXCXE	핵손 E451Q	ΔE3-RID α / β , 14.7k +EGFRVHH-FKBP FRB*-섭유	ΔE4orf6/7
AdSyn-C0442 (31)	E1A ΔLXCXE	핵손 E451Q	FRB*-섭유	ΔE4orf6/7
AdSyn-C0440 (68)	E1B-55k-P2A-Ypet	wt	ΔE3-RID α / β , 14.7k +EGFRVHH-GS-FKBP- P2A-ADP FRB*-섭유	wt

[0315] AdSyn-C0312, AdSyn-C0313 및 AdSyn-C0335는 라파마이신- 및/또는 라파로그(rapalog, AP21967)-의존적 EGFR-표적화된 바이러스이다(표적화 방법의 설명을 위하여, 참조로 본 명세서에 참조문헌으로 삽입된 PCT 공개번호 WO 2013/138505을 참조). 상기 재조합 바이러스의 각각은 E3-RID α / β 및 E3-14.7k 코딩 서열의 결실, 및 FRB*-섭유(라파마이신 또는 라파로그에 결합할 수 있는 돌연변이 FRB에 융합된 Ad5 섭유) 또는 FRB-섭유(단기 라파마이

신에 결합할 수 있는 야생형 FRB에 결합된 Ad5 섬유)의 어느 하나와 EGFRVHH-FKBP 융합 단백질의 삽입을 포함한다. AdSyn-C0442은 라파마이신/라파로그-의존적 세포표적화에 필요한, FKBP 융합 단백질이 아닌, FRB*-섬유를 발현하기 때문에, 실험 대조군 바이러스(experimental control virus)로서 사용될 수 있다. 또한, AdSyn-C0335 및 AdSyn-C0442은 핵소 단백질에서 E451Q 돌연변이를 포함한다. 이 돌연변이는 재조합 바이러스를 간으로 비표적화한다.

[0316] 도 18A은 라파마이신을 가진 FRB 도메인과 FKBP12의 결합 경계면을 나타낸다. 도 18B은 FRB-섬유(AdSyn-C0205; 서열번호 88)을 가진 EGFR-표적화 바이러스는 AP21967가 아닌, 라파마이신이 존재할 때 MDA MB 453 유방암 세포로의 형질도입이 향상되고, 반면, FRB*-섬유(AdSyn-C0220; 서열번호 98)을 가진 EGFR-표적화 바이러스는 라파마이신 또는 AP21967의 존재에서 MDA MB 453 세포로의 형질도입의 향상을 나타내는 그래프이다. 도 18C는 mTOR체의 표적 p70 S6K의 인산화를 차단하는 mTOR 저해제 라파마이신의 활성을 나타내는 면역블롯이다. 라파마이신 동족체(homolog) AP21967는 표적화 농도에서 mTOR를 저해하지 않는다.

[0317] AdSyn-C0312을 HS578T 전이성 유방암 세포에서 테스트하였다. 감염 후 24, 48, 72 및 96 시간에, 신선한 라파마이신 또는 AP21967을 50 nM로 첨가하였다. 대사 활성을 WST-1 분석으로 정량화하고, 상응하는 약물을 처리한 비감염된 세포로 정규화(normalization)하였다. 도 18D에 나타난 바와 같이, AdSyn-C0312의 감염은 표적의 부재와 비교하여 라파마이신 또는 AP21967가 처리된 EGFR-표적화 종양 살상 바이러스로 감염된 세포의 증강된 세포 살상을 초래한다. 감염 9일 후에 AdSyn-C0313-감염된 HS578T 전이성 유방암 세포의 세포 생존능을 도 18E에 나타냈다. 세포를 AdSyn-C0313의 연속 희석을 통해 감염시켰다. 감염 후 24, 48, 72 및 96 시간째에, 신선한 라파마이신을 50 nM로 첨가하였다. 대사 활성을 WST-1 분석으로 정량화하고, 상응하는 약물을 처리한 비감염된 세포로 정규화(normalization)하였다. AdSyn-C0313은 E1A ΔLXCXE, ΔE4orf6/7, ΔE3-RIDα/β, ΔE3-14.7k의 종양 살상 돌연변이를 가지고 있고 라파마이신-의존성 EGFR-표적화 유전자 EGFRVHH-FKBP 및 FRB-섬유를 발현한다. 표적화없이도 라파마이신이 처리된 EGFR-표적화 종양 살상 바이러스로 감염된 세포의 증강된 살상이 있다.

[0318] AdSyn-C0313은 HS578T를 피하 이종이식한 마우스에서 평가하였다. 종양을 가진 마우스에 AdSyn-C0313을 종양내로 주입하고, 이후 주기적으로 복강 내 8 mg/kg 라파마이신(n=5) 또는 비히클 대조군(n=4)을 투여하거나, 또는 종양내 비히클 대조군을 주입하고, 후속적으로 주기적 복강 내 8 mg/kg 라파마이신 주사 (n=5) 또는 비히클 대조군 (n=4)을 투여하였다. 감염은 초기 감염 이후 19일째에 시작해서 매 4 일마다 3회 동일한 군에 되풀이하였다. 도 19에 나타난 바와 같이, 라파마이신과 함께 EGFR-표적화 종양 살상 바이러스가 투여된 마우스의 종양은 가장 좋은 반응을 나타냈다.

[0319] AdSyn-C0335은 핵소 E451Q 돌연변이에 의해 간 흡수를 회피하는 캡시드(capsid)를 특징으로 하는 종양 살상 바이러스이고, 라파마이신 또는 라파로그 AP21967 존재에서 EGFR을 통해 세포를 감염시키도록 표적화될 수 있다. AdSyn-C0335의 종양 살상 활성을 시험관 내 및 생체 내에서 테스트하였다. HS578T 세포를 AdSyn-C0335의 연속 희석을 통해 감염시켰다. 감염 후 24, 48, 72 및 96시간째에, 신선한 라파마이신 또는 AP21967를 50 nM로 첨가하였다. 대사 활성을 WST-1 분석으로 정량화하고, 상응하는 약물을 처리한 비감염된 세포로 정규화(normalization)하였다. 도 20에 나타난 바와 같이, 표적화없이 감염된 세포와 비교하여, EGFR-표적화 종양 살상 바이러스로 감염된 라파마이신 또는 AP21967이 처리된 세포에서 증강된 세포사멸이 있다. 생체 내 AdSyn-C0335를 평가하기 위하여, 마우스에 HS578T 피하 이종이식을 마우스에 수행하였다. 종양을 가진 마우스는 4 일마다 AdSyn-C0335의 3회 종양내 주사를 받은 후, 이후 격일로 복강 내로 2 또는 8 mg/kg 라파마이신 (각각, n=8 및 n=8) 또는 비히클 대조군 (n=6)을 투여받거나; 또는 종양을 가진 마우스를 4 일마다 AdSyn-C0442의 3회 종양내 주사를 받은 후, 이후 격일로 복강 내로 2 또는 8 mg/kg 라파마이신 (각각, n=6 및 n=6)를 받거나; 또는 종양을 가진 마우스를 4일마다 비히클 대조군의 3회 종양내 주사를 받은 후, 이후 격일로 복강 내 2 또는 8 mg/kg 라파마이신(각각, n=6 및 n=6)을 투여받았다. 도 21A-21D에 나타난 바와 같이, 8 mg/kg 라파마이신과 같이 EGFR-표적화 종양 살상 바이러스를 받은 마우스의 종양은 가장 현저한 반응을 나타낸다.

[0320] AdSyn-C0199, AdSyn-C0200 및 AdSyn-C0171은 E1A 결실을 가지고 EF1α 프로모터(EF1α-루시페라제)에 의해 조절되는 루시페라제를 암호화하는 재조합 리포터 바이러스이다. 또한 AdSyn-C0200 및 AdSyn-C0171은 간에서 바이러스 유전자 발현을 억제하기 위하여, E1A의 3'-UTR에서(간 특정 마이크로 RNA) miR-122에 대한 2개의 결합 부위를 포함한다. 추가적으로 AdSyn-C0171은 핵소 단백질의 간 비표적화 E451Q 돌연변이를 암호화한다.

[0321] 도 22A-22C는 간에서 아테노바이러스 매개된 유전자 발현의 제거, 및 간으로의 아테노바이러스 감염의 비표적화를 나타낸다. AdSyn-C0199는 전신성 감염후에 오로지 간에서 높은 수준의 루시페라제를 발현하였다(도 22A). 도 22B에 나타난 바와 같이, 루시페라제 신호는, 간-특이적 마이크로 RNA miR122가 루시페라제 발현을 제거하는 것

을 제외하고는, 마우스가 AdSyn-C0199에 상응하는 바이러스, AdSyn-C0200으로 전신성으로 감염되었을 때 사라진다. 도 22C에 나타난 바와 같이, 루시퍼라제 발현은, 아데노바이러스 핵소 단백질이 간으로부터 비표적화하는 돌연변이 E451Q를 가지는 것을 제외하고, 마우스가 AdSyn-C0171을 상응하는 바이러스, AdSyn-C0171로 전신성 감염될 때 비장에서 검출될 수 있다. 상기 데이터는 Ad E1 유전자의 간에서의 발현을 방지하고 간세포에서 복제를 조절하기 위한(definition replication) 게놈 모듈 변형의 유용성을 입증한다.

[0322] 실시예 3: 키메라 섬유 단백질을 발현하는 재조합 아데노바이러스

[0323] 하기 실시예는 특정한 세포 유형에 직접 감염하는 키메라(chimeric) 섬유 단백질을 발현하는 재조합 아데노바이러스를 설명한다.

[0324] Ad5 및 많은 다른 혈청형의 섬유 단백질(fiber protein)이 세포내 부착을 위해 콕사키 아데노바이러스 수용체 (CAR)에 결합하는 것으로 알려진 반면에, 다른 혈청형은 CD46, 데스모글레인 2, 시알산, 또는 다른 것들을 이용하는 것으로 알려져 있다. Adassembly/AdSLIC(본 명세서에서 참조문헌으로 인용된, PCT 공개번호 WO 2012/024351 을 참조)는 키메라 바이러스의 신속한 제조를 허용하기 때문에, 6개의 섬유 키메라 바이러스로 구성된 패널(panel)이 다양한 혈청형의 세포내 표적화 능력을 조사하기 위해서 생성되었다. 섬유 단백질의 C-말단의 글로불라 노브(globular knob)는 수용체와의 결합에 관여하기 때문에, 키메라는 Ad5 섬유 노브를 Ad3, Ad9, Ad11, Ad12, 또는 Ad34로부터의 섬유 노브로 대체하여 제조하였다(도 23A23C; 표 3). 각각의 바이러스를 E1A/E1B 결실 및 EF1 α 프로모터에 의해 조절되는 루시퍼라제-GFP 융합체를 포함하는 동일한 E1 모듈로 제조되었다. 상기 패널(panel)을 17 다른 종양세포주, 주요 기도 세포 및 H9 줄기 세포주로 형질도입하기 위해 사용하고, 루시퍼라제 발현을 각각 세포 유형에서 측정하였다(도 23D). Ad5 섬유와 비교하여, 현저하게 높은 루시퍼라제-GFP 발현을 Ad3, Ad11 또는 Ad34를 가진 키메라를 사용할 때 대부분 모든 세포에서 관찰하였다(도 23D 및 23E). 정반대로, 루시퍼라제-GFP 발현은 Ad9 또는 Ad12 섬유 키메라로 형질도입된 세포에서 거의 보편적으로 낮았다. 상기 데이터는 Ad5-기반으로 하는 벡터를 향상시키고 특정한 세포 유형으로 감염을 위한 재조합 바이러스를 최적화하기 위해서, 다른 혈청형으로부터의 변형된 부분을 조합하는 것의 강력한 유용성을 증명한다.

[0325] 표 3. 키메라 섬유 단백질을 가진 재조합 아데노바이러스

표 3

바이러스 명칭 (서열번호)	E1	L3	E3	E4
AdSyn-C0172 (69)	Δ E1+EF1 α - 루시퍼라제-miR122	핵소 E451Q	Ad3 노브 섬유 키메라	wt
AdSyn-C0173 (70)	Δ E1+EF1 α - 루시퍼라제-miR122	핵소 E451Q	Ad9 노브 섬유 키메라	wt
AdSyn-C0174 (71)	Δ E1+EF1 α - 루시퍼라제-miR122	핵소 E451Q	Ad11 노브 섬유 키메라	wt
AdSyn-C0175 (72)	Δ E1+EF1 α - 루시퍼라제-miR122	핵소 E451Q	Ad12 노브 섬유 키메라	wt
AdSyn-C0176 (73)	Δ E1+EF1 α - 루시퍼라제-miR122	핵소 E451Q	Ad34 노브 섬유 키메라	wt

[0327] 실시예 4: 캡시드(capsid)-교환된 재조합 아데노바이러스

[0328] 하기의 실시예는 캡시드(capsid)-교환된 키메라 아데노바이러스를 만들기 위한 모듈 변형을 설명한다. 실시예에서 설명된 재조합 아데노바이러스는 존재하는 중화 항체를 회피하기 위해 디자인되었다.

[0329] 인간 집단에 존재하는 항체로부터 ‘보이지 않는(invisible)’ 효과를 제공하고, Adassembly/AdSLIC의 반복된 접종이 가능한 완전한 ‘캡시드(capsid)’ 모듈 교환(거의 60%의 게놈)을 완성한 Ad5-기반으로 하는 바이러스는 아데노바이러스 게놈에 존재하는 모듈성(modularity)을 이용하도록 디자인되었다. 상기 모듈성의 가장 팔팔한 테스트는 하나의 혈청형의 전체 모듈을 다른 혈청형의 전체 모듈과 결합하는 것일 수 있다. 이러한 가능성은 Ad5의 E1, E3, 및 E4 모듈을 다른 혈청형의 코어 모듈(즉, E2B, L1, L2, L3, E2A 및 L4; 도 24A 참조)과 결합함으로써 조사되었다. 이는 중화효과를 나타내는 Ad5 항체를 회피할 수 있는 바이러스를 생산하나, 종양 살상 바이러스를 제조할 때 빈번히 변이되는 Ad5의 잘 분석되어 있는 비-구조적 유전자를 유지한다. AdSLIC는 Ad3, Ad9, Ad11, Ad12, Ad34 또는 Ad5의 E1, E3, 및 E4 모듈을 가지는 마우스 아데노바이러스 1 (MAV-1)의 코어 모듈이

포함된 바이러스를 생성하는데 사용었다(도 24B 및 표 4). 추가적으로, Ad5 E1 모듈은 Ad5의 pIX 코딩 영역을 상응하는 코어 혈청형의 pIX 코딩 영역으로 치환 변경되었고; 및 Ad5 E3 모듈은 U엑손 및 섬유 코딩 영역을 상응하는 코어 혈청형의 것으로 치환 변형되었다. 따라서, 모든 캡시드(capsid) 성분은 하나의 혈청형으로부터인 반면, 게놈의 E1, E3, 및 E4 영역은 Ad5로부터이다. 만들어진 6개의 키메라 중 4개는 293-E4 세포에서 생존가능한 바이러스를 생산할 수 있다. Ad5와 Ad3, Ad9, Ad11, 또는 Ad34 사이의 키메라는 모두 복제한 반면, Ad5/Ad12 또는 Ad5/MAV-1 키메라는 ITRs 및 코어 혈청형의 포장 서열 (Ψ)을 포함하도록 추가 변형된 후에도 복제하지 않았다(도 24B). 따라서, 상기 “캡시드-교환된” 아데노바이러스 키메라를 생성하기 위한 능력은 혈청형에 따라 다양하다.

[0330] 표 4. 캡시드(capsid) 교환을 가진 재조합 아데노바이러스

표 4

[0331]

바이러스 명칭 (서열번호)	E1	코어	E3	E4
AdSyn-C0159 (74)	Ad11 pIX	A11 Ad11	Ad11 U엑손 및 섬유	wt
AdSyn-C0159X (93)	Ad11 pIX E1A Δ LXCXE	A11 Ad11	Ad11 U엑손 및 섬유	Δ E4orf6/7
AdSyn-C0167 (75)	Ad9 pIX	A11 Ad9	Ad9 U엑손 및 섬유	wt
AdSyn-C0167X (94)	Ad9 pIX E1A Δ LXCXE	A11 Ad9	Ad9 U엑손 및 섬유	Δ E4orf6/7
AdSyn-C0168 (76)	Ad12 pIX	A11 Ad12	Ad12 U엑손 및 섬유	wt
AdSyn-C0201 (77)	Ad3 pIX	A11 Ad3	Ad3 U엑손 및 섬유	wt
AdSyn-C0201X (95)	Ad3 pIX E1A Δ LXCXE	A11 Ad3	Ad3 U엑손 및 섬유	Δ E4orf6/7
AdSyn-C0202 (78)	Ad34 pIX	A11 Ad34	Ad34 U엑손 및 섬유	wt
AdSyn-C0202X (96)	Ad34 pIX Δ E1A Δ LXCXE	A11 Ad34	Ad34 U엑손 및 섬유	Δ E4orf6/7
AdSyn-C0185 (79)	MAV-1 pIX E1 +CMV-GFP	A11 MAV-1	MAV-1 U엑손 및 섬유	wt
AdSyn-C0254 (80)	Ad12 ITR/ Ψ , Ad12 pIX	A11 Ad12	Ad12 U엑손 및 섬유	Ad12 ITR
AdSyn-C0255 (81)	MAV-1 ITR/ Ψ , MAV-1 pIX Δ E1 +CMV-GFP	A11 MAV-1	MAV-1 U엑손 및 섬유	MAV-1 ITR

[0332]

생존한 캡시드(capsid) 교환된 키메라 바이러스의 복제 및 유전자 발현을 후속적으로 분석하였다. 정제된 캡시드(capsid) 교환된 입자는 상응하는 코어 모듈을 가진 야생형 아데노바이러스 혈청형과 동일한 총 단백질 함량을 나타냈다(도 25A). 대다수의 중화 항체의 표적인 핵산 단백질은 Ad5 입자에 대하여 생성된 다클론 래빗 항혈청에 의해 다른 혈청형 또는 캡시드 교환된 바이러스에서 인식되지 않았다(도 25A). V 및 VI와 같은 캡시드의 내부에서 발견된 다른 구조적인 단백질은 일부 교차 반응성(cross reactivity)을 나타낸다(도 25A). 감염된 세포의 웨스턴 블랏 분석은, Ad5/Ad3, Ad5/Ad11, 및 Ad5/Ad34 키메라에 대하여, 교차 반응하는 후기(late) 단백질 및 100K의 발현이 A549 세포에서 야생형 Ad3, Ad11, 및 Ad34와 동일한 반면, U2OS 세포에서는 약간 높은 것을 나타냈다(도 25B, 25D 및 25E). 모든 바이러스에서 Ad5로부터 유래된 E1A의 발현은, 아마도 Ad5에 대한 향상된 세포내 침투(entry)에 기인하여, 상기 3개의 키메라에서 야생형 Ad5보다 현저하게 높았다(도 25B, 25D 및 25E 및 도 23D). 정반대로, Ad5/Ad9 캡시드 교환된 키메라는, 야생형 Ad9(late 단백질에 대하여)보다 약간 나쁘게 야생형 Ad5보다 나쁘고(E1A에 대하여), 동일한 MOI에서 낮은 유전자 발현을 나타냈다(도 25C).

[0333]

다음으로, 캡시드 교환된 키메라 바이러스가 Ad5 중화 항체를 포함하는 인간 혈청 시료에서 항체 중화를 회피할 수 있는지를 테스트하였다. 인간 혈청 시료는 WST-1 세포 생존능 분석을 사용하여 측정된 바와 같이, Ad5에 의

한 A549 세포의 살상을 증화시킬수 있음이 확인되었다. Ad5 또는 캡시드 교환된 키메라와 상기 혈청 시료를 배양했을 때, Ad5에 의한 세포 사멸은 억제된 반면, 캡시드 교환된 바이러스에 의한 살상은 영향을 받지 않았다(도 26A-26C). 상기 데이터는 캡시드 교환된 키메라 바이러스는 인간 시료에서 통상적으로 발견되는 기존의 Ad5 항체를 회피할 수 있다는 것과 Ad5의 비-구조적 성분 및 비-Ad5의 구조적 성분의 발현과 함께 복제할 수 있다는 것을 나타낸다. 따라서, 아데노바이러스 게놈 내에서 관찰된 천연 모듈성은 유용한 성질을 가진 신규 바이러스를 만들기 위하여 Adsembly/AdSLIC를 사용하여 개발될 수 있다.

[0334] 상기 논의된 바와 같이, 인간 집단의 상당비율은 Ad5 및 일부 다른 통상 아데노바이러스 혈청형에 대해 항체를 가진다. 16명의 인간 혈청 시료의 분석은 16개의 시료 중 9개가 Ad5에 대한 중화 항체를 포함하고 있음을 나타냈다. 따라서, 기존의 중화 항체를 회피할 수 있는 캡시드 교환을 가진 종양 살상 바이러스가 개발되었다. E1A(Δ LXCXE) 및 E4(Δ E4orf6/7)에 종양 살상 돌연변이가 포함된 4개의 재조합, 캡시드-교환된 아데노바이러스가 제조되었다. 상기 돌연변이는 AdSyn-C0181에 존재하는 것과 동일한 종양 살상 돌연변이이다. AdSyn-C0159X(서열번호 93), AdSyn-C0167X(서열번호 94), AdSyn-C0201X(서열번호 95) 및 AdSyn-C0202X(서열번호 96)는 각각 Ad11, Ad9, Ad3 및 Ad34로부터의 구조적 단백질을 포함한다.

[0335] **실시예 5: 변형된 또는 키메라 섬유 단백질, 또는 캡시드(capsid) 돌연변이 또는 혈청형 교환을 결합시킨 재조합 종양 살상 아데노바이러스**

[0336] 본 실시예는 종양 선별력, 및/또는 특정한 세포 유형으로 직접적 감염을 가능하게 하는 돌연변이 또는 키메라 섬유 단백질을 발현하는, 및/또는 중화 항체를 회피하기 위해 디자인된 캡시드-변형되거나 또는 교환된 키메라 아데노바이러스를 가진 추가 재조합 아데노바이러스를 설명한다. 대표적 재조합 바이러스를 표 5에 열거하고 이들 바이러스의 게놈 서열은 서열번호 82-87로 기재하였다.

[0337] **표 5. 변형된 또는 키메라 섬유 단백질, 또는 캡시드 돌연변이 또는 혈청형 교환을 결합시킨 재조합 종양 살상 아데노바이러스**

표 5

바이러스 명칭 (서열번호)	E1	코어	E3	E4
AdSyn-C0507 (82)	E1A Δ LXCXE	핵손 E451Q	Ad34 노브 섬유 키메라	Δ E4orf6/7
AdSyn-C0508 (83)	E1A Δ LXCXE	핵손 E451Q	Ad11 노브 섬유 키메라	Δ E4orf6/7
AdSyn-C0509 (84)	E1A Δ LXCXE	핵손 E451Q	Ad37 노브 섬유 키메라	Δ E4orf6/7
AdSyn-C0510 (85)	E1A Δ LXCXE/Ad34 pIX	모든 Ad34	Ad34 U엑손 및 섬유	Δ E4orf6/7
AdSyn-C0511 (86)	E1A Δ LXCXE	핵손 E451Q/펜톤 RGE	Δ E3-RID α / β , Δ E3-14.7k +EGFRVHH-FKBP FRB*-섬유	Δ E4orf6/7
AdSyn-C0512 (87)	E1A Δ LXCXE	핵손 E451Q/펜톤 RGE	섬유 HI 루프에 있는 RGD-4C	Δ E4orf6/7

[0339] 여기에(표 1-4 참조) 개시된 각각의 재조합 아데노바이러스는 존재하는 중화 항체를 회피하고 특정한 세포 유형으로 감염을 유도하는데 유리한 변형을 가진다. 실시예 1에서 설명된 E1 및 E4 돌연변이는 종양 살상 바이러스 치료의 효능을 강화시키기 위해 다른 모듈과 결합될 수 있다.

[0340] 키메라 섬유와 같이 E1, 코어, 및 E4 돌연변이를 가진 재조합 종양 살상 아데노바이러스의 예는 각각 d34, Ad11, 및 Ad37 섬유 단백질의 노브(knob) 도메인을 사용하는, AdSyn-C0507, AdSyn-C0508, 및 AdSyn-C0509가 있다.

[0341] Ad34 캡시드 교환을 함께 E1 및 E4 돌연변이를 가지는 재조합 종양 살상 아데노바이러스의 한 예는 AdSyn-C0510이다.

- [0342] RGE에 대한 인테그린-결합 RGD 모티프에서 돌연변이를 가지는 캡시드 단백질 펜톤과 함께 E1, E3, 및 E4 돌연변이를 가지고, 라파마이신/라파로그 유도성 EGFR-표적화 종양 살상 아데노바이러스의 한 예는 AdSyn-C0511이다. 상기 펜톤 돌연변이는 비장에서 바이러스 흡수를 감소시키고 항바이러스 염증 반응을 약화시킨다.
- [0343] 섬유 단백질에서 RGD 펩티드의 삽입과 함께 E1 및 E4 돌연변이를 가진 재조합 종양 살상 아데노바이러스의 한 예는 AdSyn-C0512이다. 섬유 단백질에서 RGD 삽입은 혈관내피세포(vascular endothelial cell)를 포함하여, 넓은 다양한 세포 유형으로의 감염을 현저하게 증가시키는 것으로 확인되었다.
- [0344] **실시예 6: Ad5 및 다른 아데노바이러스 혈청형으로부터 유래된 종양 살상 아데노바이러스**
- [0345] 여기에(표 1-5 참조)는 Ad5 벡터를 사용하여 개발되었다. 그러나, 아데노바이러스 복제에 중요한 많은 게놈영역(및 이 영역에서 암호화는 단백질)은 아데노바이러스 혈청형 간에 고도로 보존되었다. 따라서, 여기서 개시된 재조합 아데노바이러스는 임의의 원하는 아데노바이러스 혈청형으로부터 게놈 서열을 사용하여 생성될 수 있다. 특히, E1A 및 E4orf6/7 단백질은 모든 인간 종의 아데노바이러스 간에 보존되어 왔다. 도 27은 종(species) A (Ad12), 종 B (Ad7), 종 C (Ad2 및 Ad5), 종 D (Ad9), 종 E (Ad4), 종 F (Ad40) 및 종 G (Ad52) 아데노바이러스로부터 E1A 단백질의 정렬을 나타낸다. 유사하게, 도 28은 종 A (Ad12), 종 B (Ad7), 종 C (Ad2 및 Ad5), 종 D (Ad9), 종 E (Ad4) 및 종 G (Ad52) 아데노바이러스의 E4orf6/7 단백질들의 정렬(alignment)을 나타낸다. 도 27 및 28에 나타난 서열, 공공 데이터베이스에서 사용가능한 임의의 다른 아데노바이러스 서열을 사용하여, 종양 살상 아데노바이러스의 생성에 임의의 아데노바이러스 혈청형 유래의 E1A 및/또는 E4orf6/7 서열을 쉽게 사용할 수 있다.
- [0346] **실시예 7: EGFR-표적화 요소를 가지는 재조합 아데노바이러스 특징**
- [0347] 본 실시예는 EGFR을 발현하는 세포로 표적화된 합성 아데노바이러스를 기술하며, 그 재조합 바이러스는 변형의 결과로서 293-E4 세포에서 복제 손상(replication implication)이 일어나지 않음을 확인한다. 하기의 합성 아데노바이러스는 본 연구에서 생성되고 사용되었다:
- [0348] **AdSyn-C0170 (서열번호 91)**
- [0349] 아데노바이러스 게놈 모듈로부터 다중부위 게이트웨이® LR 재조합 반응인, “에드셈블리(Adsembly)”를 사용하여 만든 야생형 아데노바이러스. 3개의 재조합 부위는 아데노바이러스 모듈-E1B-55K 및 pIX 유전자 사이의 attB4 재조합 부위; L4-33K 및 pVIII 유전자 사이의 attB5 부위; 및 섬유 및 E4 유전자 사이의 attB3 부위-사이의 게놈에 존재한다. 상기 재조합 아데노바이러스는 대조군 바이러스로서 역할을 한다.
- [0350] **AdSyn-C0205 (서열번호 88)**
- [0351] AdSyn-C0170에 상응하지만, 라파마이신-의존적으로 표적화가 EGFR을 발현하는 세포로의 감염을 가능하게 하는 추가적 특징을 가진, Adsembly에 의해 제조된 재조합 아데노바이러스. mTOR로부터 FRB 도메인을 암호화하는 서열을 아데노바이러스 섬유의 노브 도메인의 HI 루프에 삽입된다. E3-RID α , E3-RID β , 및 E3-14.7K 유전자는 제거되고, FKBP12에 융합된 EGFR-결합 단일-도메인 항체(EGFRVHH)를 암호화하는 유전자로 대체되었다. 라파마이신의 존재에서, 가용성 EGFRVHH-FKBP 융합 단백질은 섬유에서 FRB 도메인과 헤테로다имер(heterodimer)를 형성하고, 성숙한 아데노바이러스 입자가 새로운 세포 상의 EGFR에 결합하여 그 새로운 수용체를 경유하여 감염시킬 수 있도록 한다.
- [0352] **AdSyn-C0206 (서열번호 89)**
- [0353] 재조합 아데노바이러스가 야생형 섬유를 가지는 것, 따라서 FRB 도메인이 없는 것을 제외하면, AdSyn-C0205와 동일하다. 상기 바이러스는 AdSyn-C0205에 설명된 바와 같이, E3-RID α , E3-RID β , 및 E3-14.7K 유전자의 결실, 및 FKBP12에 융합된 EGFR-결합 단일-도메인 항체 (EGFRVHH)를 암호화하는 유전자의 치환이라는 특성을 가진다.
- [0354] **AdSyn-C0207 (서열번호 90)**
- [0355] 재조합 아데노바이러스는, 야생형 E3 영역을 가지는 것, 따라서 EGFRVHH-FKBP 융합체가 없는 것을 제외하고는, AdSyn-C0205와 동일하다. AdSyn-C0205에서 설명된 바와 같이, 이것은 섬유에 FRB 삽입이라는 특징을 가진다.
- [0356] **AdSyn-C0220 (서열번호 98)**
- [0357] 재조합 아데노바이러스는, 라파마이신 또는 AP21967으로 EGFR-표적화를 가능하게 하는, FRB 도메인 (FRB*)에서

T2098L 돌연변이를 가지는 것을 제외하고는, AdSyn-C0205와 동일하다.

- [0358] 라파마이신에 의해 조절된 EGFR-표적된 바이러스 AdSyn-C0205는, 감염의 마커로서 E1A에 융합된 GFP 리포터를 포함한다. E3B 전사체는 RID α , RID β , 및 14.7k를 삭제하여 변형되고, EGFRVHH-FKBP를 발현하는데 사용된다. AdSyn-C0206은 AdSyn-C0205의 모든 변형을 가지나, 야생형 섬유를 유지하므로, FRB 도메인을 가지지 않는다. AdSyn-C0207은 AdSyn-C0206의 모든 변형을 가지나, 야생형 E3 영역을 유지하므로, FKBP-융합체 유전자를 가지지 않는다.
- [0359] 상기 재조합 바이러스의 Ad5 후기(late) 단백질 발현을 조사하기 위하여, 293-E4 세포를 MOI 10의 “야생형” 대조군 바이러스 AdSyn-C0170, 변형된 라파마이신-의존적 EGFR-표적화 바이러스 AdSyn-C0205, 또는 대조군 바이러스 AdSyn-C0206 또는 AdSyn-C0207로 감염시켰다. 도 29는 감염된 293-E4 세포의 용해물로부터 단백질 발현의 면역블롯을 보여준다. 섬유 이동의 변화는 AdSyn-C0207 및 AdSyn-C0205에서 90개의 아미노산 FRB 도메인 삽입을 나타낸다. EGFRVHH-FKBP 융합 단백질의 발현을 FKBP12에 대한 프로빙(probing)으로 검출하였다. 시간경과에 따른 후기(late) 단백질 발현 결과는 Adassembly 야생형 AdSyn-C0170와 비교하여 캡시드-돌연변이, 또는 GFP-E1A 리포터를 가진 바이러스를 발현하는 EGFRVHH-FKBP의 복제에서 확인한 결함이 없음을 보여준다.
- [0360] **실시예 8: AdSyn-C0335의 라파마이신-유도된 EGFR-표적화는 마우스에서 HS578T 이종이식의 종양 살상 치료의 효능을 증가시킨다**
- [0361] 본 실시예는 재조합 아데노바이러스의 라파마이신-의존적 EGFR 재표적화가 EGFR-발현하는 종양 이종이식의 종양 부피를 현저하게 감소시키는 사실을 설명한다.
- [0362] **마우스 및 종양**
- [0363] HS578T (ATCC-HTB-126; American Type Culture Collection, Manassas, CA) 유방암 세포는 공급자의 권고한대로 배양되었다. 암컷 5-주령 아쓰믹(arthymic) 마우스(J:NU #007850; Jackson Laboratory, Bar Harbor, ME)를 승인된 프로토콜하에서 사육하였다. 이종이식을 마취 상태(Isoflurane)에서 왼쪽 및 오른쪽 옆구리로 HS578T 세포 (5E6 세포 0.2 ml의 BD Matrigel Matrix에 현탁); BD Biosciences, Bedford, MA)를 피하 주입하여 개시하였다.
- [0364] 2개의 수직의 종양 지름(l 및 w)을 종양 진행을 따라 매주 측정하였다. 종양 부피를 변형된 타원체 식 $vol = 1/2(l * w^2)$ 을 사용하여 계산하였다(Euhus *et al.*, *J Surg Oncol* 31(4):229-2341986; Tomayko *et al.*, *Cancer Chemother Pharmacol* 24(3):148-154, 1989).
- [0365] 이식 후 2개월째에, $270 \pm 17 \text{ mm}^3$ (평균 $\pm$ SEM)의 부피를 가진 86개의 종양을 가진 48 마리 마우스를 본 연구분석에 포함하였고 유사한 평균-크기 종양($n=6$ 내지 8)을 가진 치료군들로 무작위로 분류하였다. HS578T의 모든 주입이 종양을 발생시키지는 않았다. 0, 3, 및 6일째에 마우스를 50 μl PBS로 희석된 2×10^8 PFU의 아데노바이러스는 마우스의 종양내로 투여되었다. AdSyn-C0335은 FRB 서열을 가진 섬유 단백질을 발현하고 EGFR(EGFRVHH)를 인식하는 나노체(nanobody)에 융합된 FKBP를 발현하는 종양 살상 아데노바이러스이다. AdSyn-C0442는 AdSyn-C0335의 모든 특성을 가지고 있으나, EGFRVHH를 발현하지 않는다. 100 μl 의 5% 트윈 80/5% PEG400에 희석된 라파마이신 (Lot ASC-127; LC Laboratories, Woburn, MA)을 2 또는 8 mg/kg 복용량으로 1, 4, 7일째에 복강내로 이후 격일로 복강 내 주사되었다. 독성에 대하여 체중을 모니터링하고, 치료군에 대하여 블라인드로 종양 크기를 측정하였다. 종양이 임의의 치수(dimenssion)에서 20 mm 이상 되거나 또는 현저한 종양 부하(tumor burden)의 다른 징후를 나타내면 마우스를 희생시켰다.
- [0366] **통계 분석**
- [0367] 통계 분석을 통계방법의 NIST/SEMATECH e-핸드북의 지침대로 R 통계 소프트웨어 3.2.0(The R Foundation, Vienna, Austria)를 사용하여 수행하였다. 이상값(outliei)은 Q-Q 플롯(plot)에 의해 시각적으로 검증된 정규 분포를 가정하고 95% 신뢰 구간에서 ESD(extream studentized deviate) 과정을 사용하여 확인되었다. 각 시점에서 수집된 치료군의 평균 종양 부피간의 차이정도는 분산 분석(ANOVA)를 이용하여 분석하였다.
- [0368] **결과**
- [0369] 생체 내 종양을 치료하기 위한 EGFR을 통한 아데노 바이러스 자손의 표적화 정도를 확인하기 위해, 피하 HS578T 이종이식이 nu/nu 마우스의 양쪽 옆구리에 수행되었다. 종양이 $264 \pm 16 \text{ mm}^3$ (평균 $\pm$ SEM)에 도달했을 때, 마우스는 비슷한 평균 크기의 종양을 가진 치료 군들로 무작위로 분류되었다. 마우스는 조양내로 mock(단지 비히

클), AdSyn-C0335 또는 AdSyn-C0442를 투여받았다. AdSyn-C0442는 AdSyn-C0335의 특성을 가지나, EGFRVHH-FKBP를 발현하지 않는 대조군 바이러스이다. 바이러스 자손의 EGFR-표적화를 위하여 치료 과정 기간에 격일로 라파마이신(rap)을 공동-투여하였다. 결과를 도 30에 나타냈다.

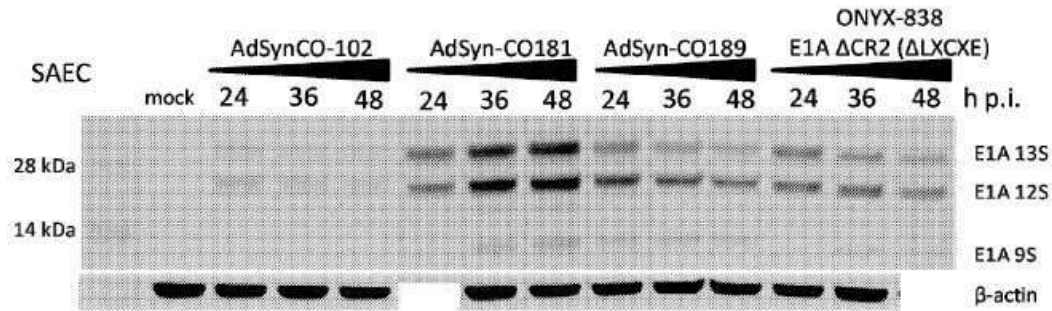
- [0370] mock 처리 및(n = 8 종양) 라파마이신 무처리군에서, 종양은 mock 처리시작 후 28일째에 $1300 \pm 448 \text{ mm}^3$ 평균 종양 부피로 자랐으며, 이후, 큰 종양 치수 때문에 마우스를 희생하였다. 비교를 위해, 하기에 기술된 데이터는 치료 과정에서 이 시점 이후 부터이다.
- [0371] 아데노바이러스 투여와 함께 격일로 8 mg/kg 라파마이신의 용량을 처음 테스트하였으나, 높은 라파마이신 농도는 바이러스 감염에 대한 효능을 상쇄시켰다. 8 mg/kg 라파마이신 단독 투여(바이러스 감염 없음)는 비처리(P = 4.50×10^{-2})한 것과 비교하여 종양 성장 정도를 늦추는데, $229 \pm 49 \text{ mm}^3$ 의 평균 종양 부피를 초래하였다. 2 mg/kg 라파마이신 단독(n = 8)의 투여는 종양 성장을 차단하는데 충분하지 않았다(P=0.144).
- [0372] 종양 살상 AdSyn-C0335 단독(n = 11)은 비처리와 비교하여 종양 성장의 비율에 영향을 주지 않았고, $948 \pm 207 \text{ mm}^3$ 의 평균 종양 부피를 나타냈다. 이는 비표적화된 AdSyn-C0335 자손에 의해 HS578T 감염의 제한에 기인한 것 같다. AdSyn-C0335 및 2 mg/kg 라파마이신 모두가 공동-투여되었을 때(n = 11), 바이러스 단독(P = 8.54×10^{-4}) 또는 2 mg/kg 라파마이신 (P = 1.49×10^{-2}) 치료 단독과 비교하여 종양 부피에서 급격하고 현저한 감소가 있었고, $133 \pm 22 \text{ mm}^3$ 의 감소된 평균 종양 부피를 나타냈다. 2 mg/kg 라파마이신 및 대조군 바이러스 AdSyn-C0442(n = 9)는, 2 mg/kg 라파마이신 치료 단독과 비교하여 평균 종양 부피의 현저한 (P = 0.449) 차이를 가지지 않고, 2 mg/kg 라파마이신 및 AdSyn-C0335의 공동 투여보다 현저한(P = 4.92×10^{-3}) 거대 종양을 나타내는, $401 \pm 89 \text{ mm}^3$ 의 평균 종양 부피를 초래하기 때문에, 상기 데이터는 종양 부피의 감소가 바이러스 자손의 라파마이신-유도된 EGFR-표적화를 의존된다는 것을 제시한다.
- [0373] 2 mg/kg 라파마이신 치료와 2 mg/kg 라파마이신 및 AdSyn-C0335의 공동-투여 사이의 현저한 차이는 치료 시작 후 빠르면 14 일에 측정되었다(P = 2.25×10^{-2}). 상기 시작점에서, AdSyn-C0442로 감염된 라파마이신-처리된 종양은 AdSyn-C0335-감염된 종양보다 더 자랐다(P = 1.54×10^{-2}).
- [0374] 본 연구는 아데노바이러스 재표적에 충분한 라파마이신 용량이 면역 억제인지를 평가하기 위하여 수행되었다. 피하 HS578T 이종이식 종양을 가진 마우스는 100 μl 비히클, 2 mg/kg 라파마이신 또는 8 mg/kg 라파마이신의 복강 내 주사로 처리되었다. 주사 후 1 시간, 이종이식 조직을 수득하고, 10% 포르말린으로 고정하고, P70S6K의 인산화-T378 형태를 인식하는 항체로 IHC 염색을 위해 섹션하였다. 도 31은 트레오닌 389에서 인산화된 P70S6K(P70S6K p-T389)에 대하여 염색된 HS578T 이종이식 조직 섹션의 미세사진(micropictographs)을 나타낸다. T389에서 P70S6K의 인산화는 mTOR 활성의 특징이다. 비처리 또는 낮은, 아데노바이러스 EGFR-표적화 유효량(2 mg/kg)으로 처리된 조직에서, P70S6K p-T389의 염색으로 입증된 바와 같이, mTOR 활성은 억제되지 않았다. 더 높은 아데노바이러스 EGFR-표적화 유효량(8 mg/kg)에서, P70S6K p-T389 염색 손실이 입증하는 바와 같이, mTOR 활성이 억제되었다. 상기 결과는 생체 내 재조합 아데노바이러스의 EGFR-표적화에 충분한 라파마이신 용량이 mTOR를 억제하지 않는다는 것을 나타낸다(따라서 대체적으로 면역억제가 아니다)
- [0375] **실시예 9: Ad34 캡시드를 가진 재조합 종양 살상 아데노바이러스**
- [0376] AdSLIC에 만들어진 아데노바이러스, AdSyn-C0335B-K (서열번호 92)는 Ad5의 모든 구조적 단백질이 Ad34 구조적 단백질로 대체되는 것을 제외하고, AdSyn-C0335 (서열번호 30)와 동일하다. 이러한 바이러스는 AdSyn-C0202처럼 동일한 캡시드(capsid) 교환을 가진다. 이는 바이러스가 AdSyn-C0335의 종양 살상 선별력을 유지하는 것을 허용하나, Ad5가 항체를 중화시키는 것은 회피한다. Ad34 섬유 노브 도메인은 Ad5 섬유 노브로 대체되었고, 노브 도메인의 HI 루프로 삽입된 mTOR로부터의 FRB 도메인을 암호화하는 서열을 가진다. 3-RID α , E3-RID β , 및 E3-14.7K 유전자를 제거하고, FKBP12에 융합된 EGFR-결합 단일-도메인 항체(EGFRVHH)를 암호화하는 유전자로 대체되었다. 라파마이신의 존재하에서, 가용성 EGFRVHH-FKBP 융합 단백질은 섬유에서 FRB 도메인을 가진 이종이량체(heterodimer)를 형성하고, 새로운 세포 상에서 EGFR에 결합하고 새로운 수용체를 통하여 감염시키는 성숙 아데노바이러스 입자를 허용한다. Ad34 섬유의 샤프트 도메인이 Ad5 샤프트에 의해 대체되는 것을 제외하고, AdSyn-C0335B-TK(서열번호 97)은 AdSyn-C0335B-K와 동일한 재조합 아데노바이러스이다.
- [0377] 본 개시의 원칙이 적용될 수 있는 많은 실시예의 관점에서, 설명된 실시예는 본 개시의 단지 실시예임을 인식해

야만 하고 본 개시의 범위를 제한하는 것으로 간주되어서는 안된다. 다만, 본 개시의 범위는 하기의 청구항에 의해 정의된다. 따라서 우리는 이들 청구항의 범위 및 정신 내에 있는 모든 것을 청구한다.

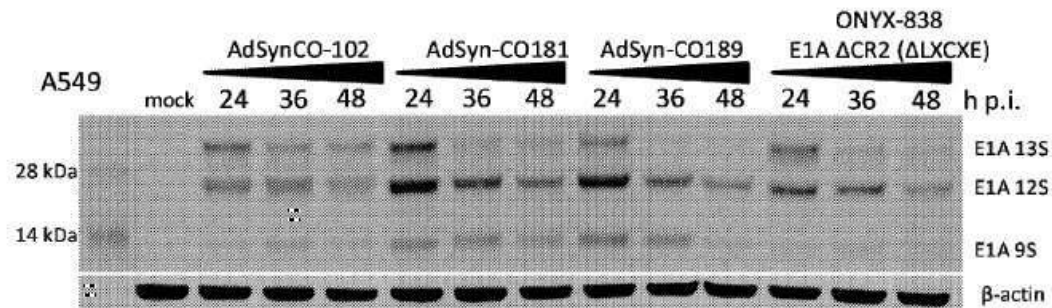
도면

도면1

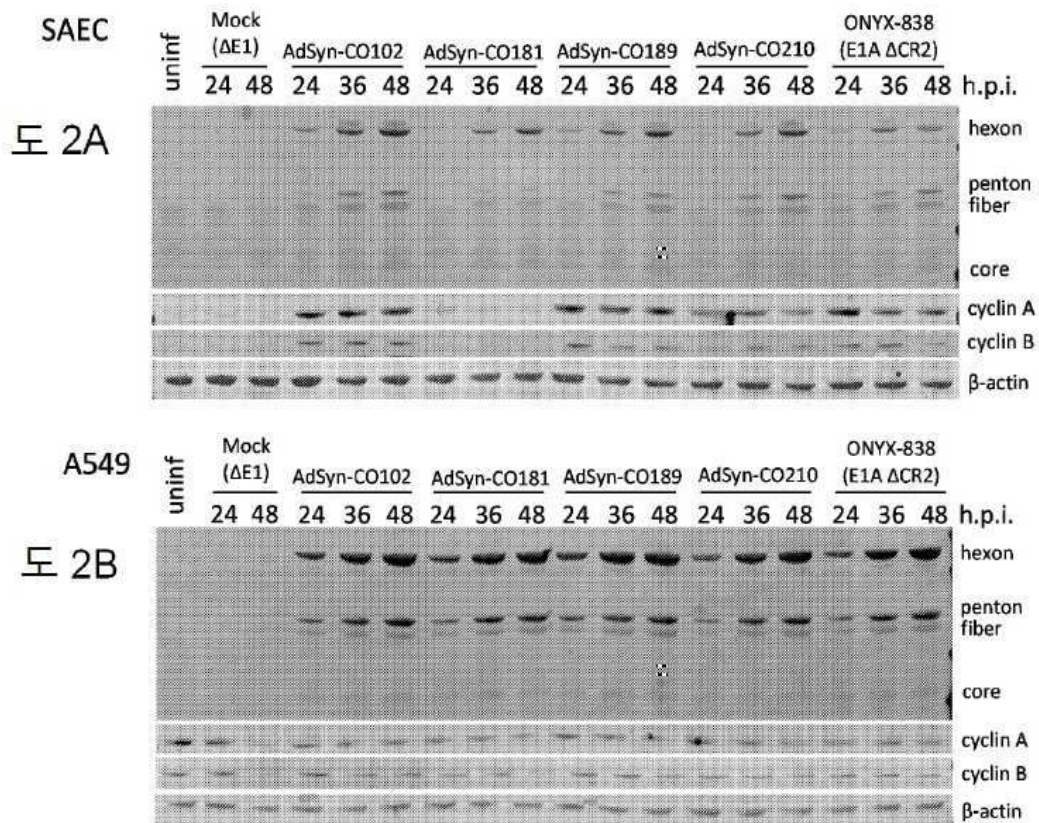
도 1A



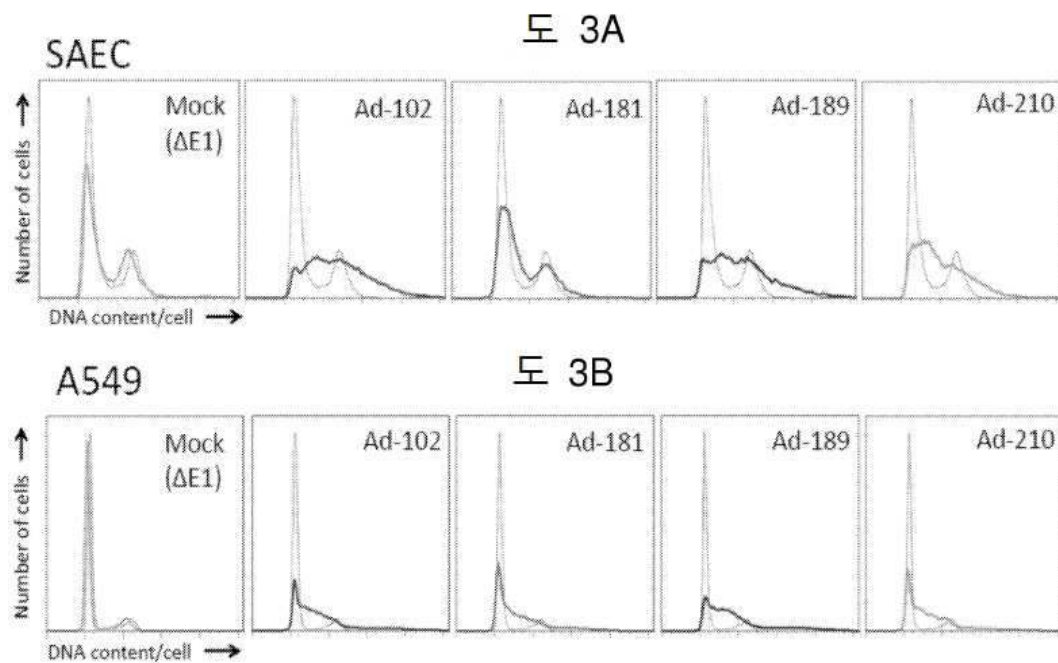
도 1B



도면2



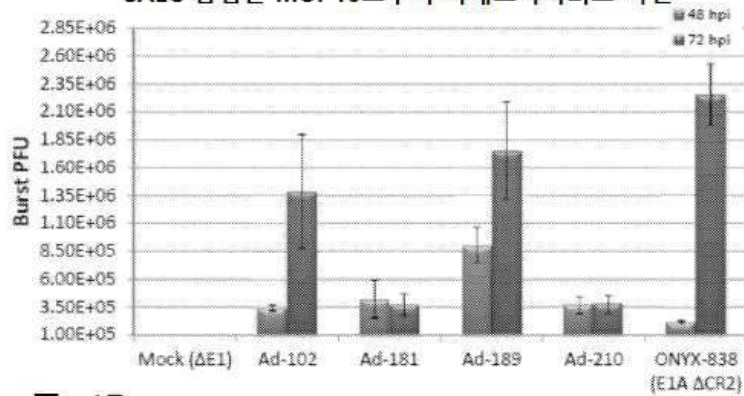
도면3



도면4

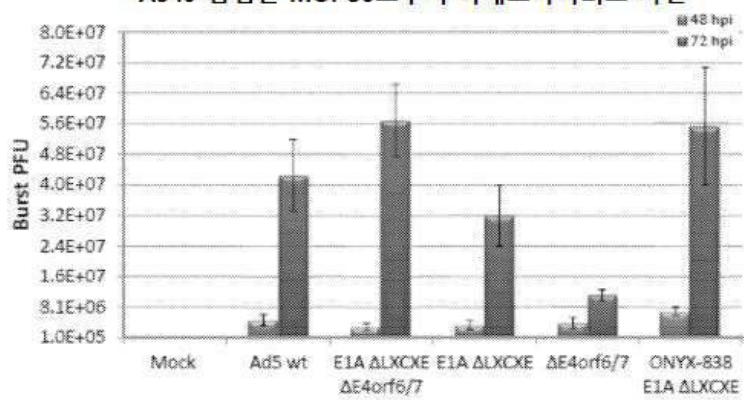
도 4A

SAEC 감염된 MOI 10로부터 아데노바이러스 파열



도 4B

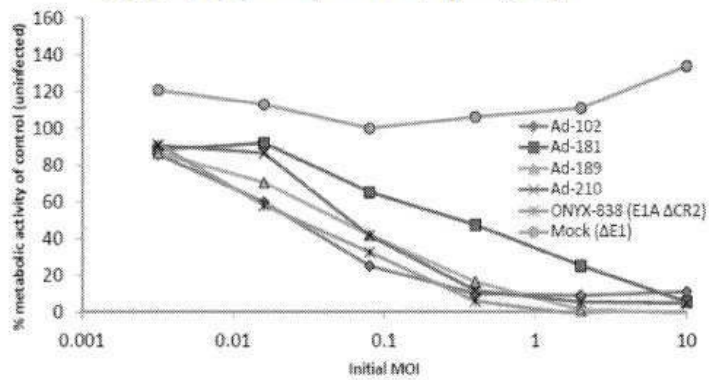
A549 감염된 MOI 30로부터 아데노바이러스 파열



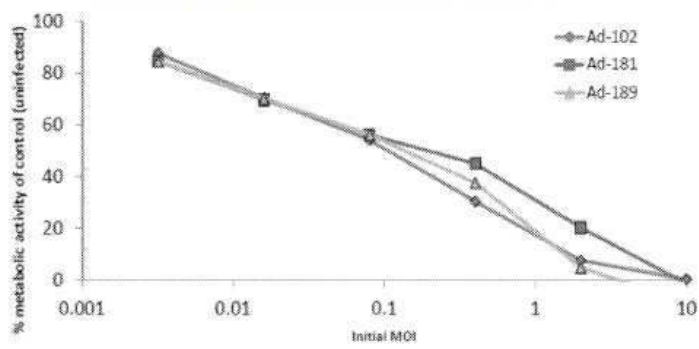
도면5

도 5A

감염된 휴지기 SAEC, 7 d.P.I.의 세포 생존력



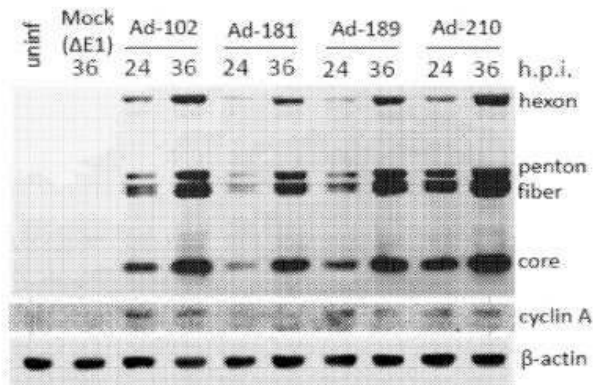
감염된 융합 A549, 7 d.P.I.의 세포 생존력



도 5B

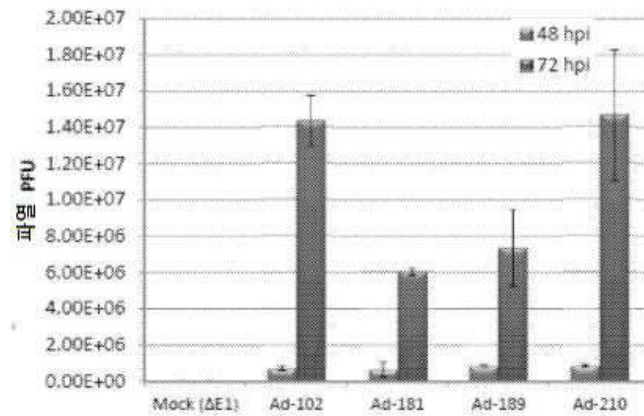
도면6

도 6A



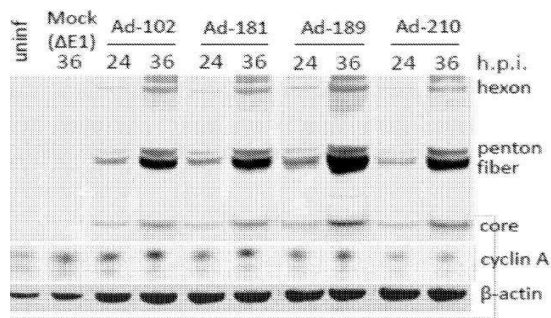
도 6B

NHA MOI 1 감염으로부터 아데노바이러스 파열



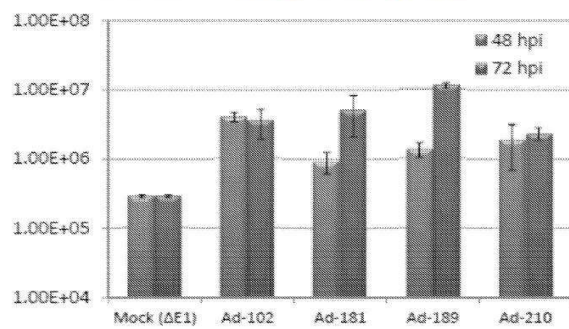
도면7

도 7A

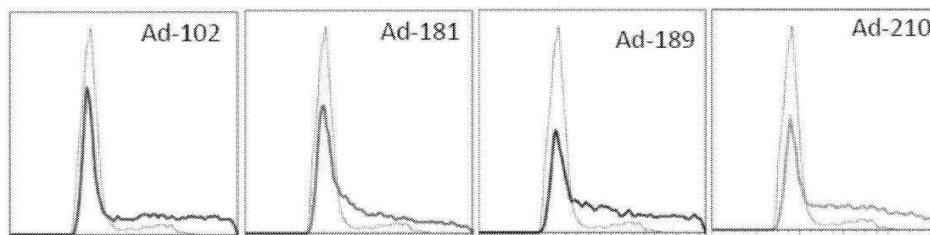


도 7B

U87 MOI 1 감염으로부터 파열

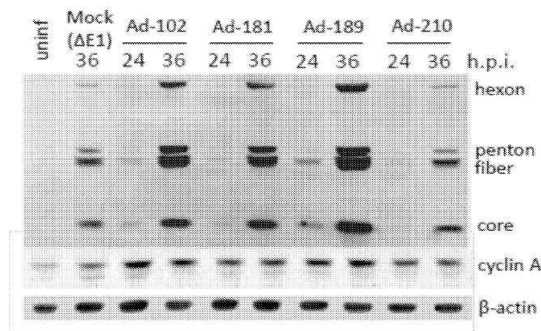


도 7C

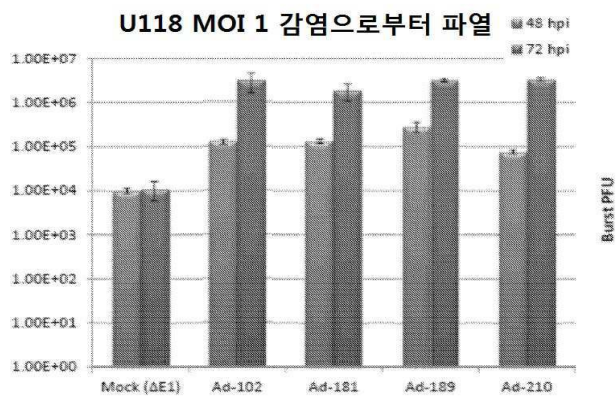


도면8

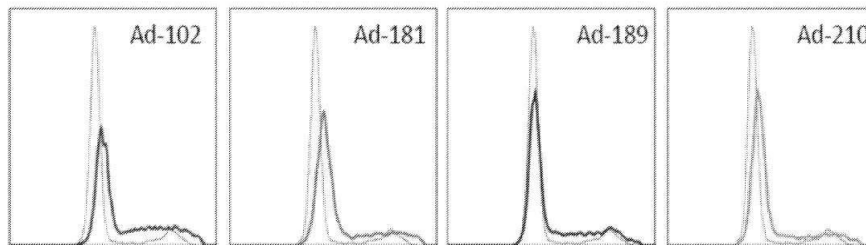
도 8A



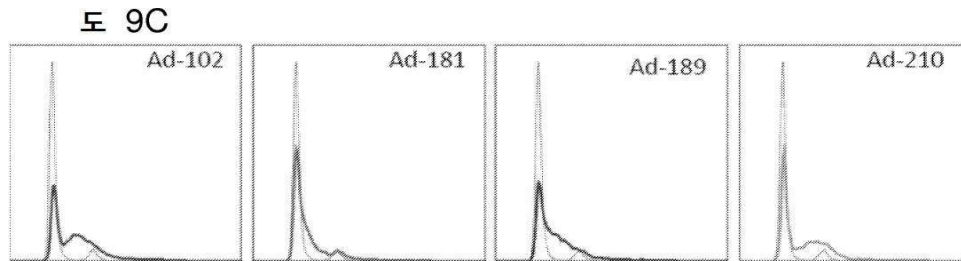
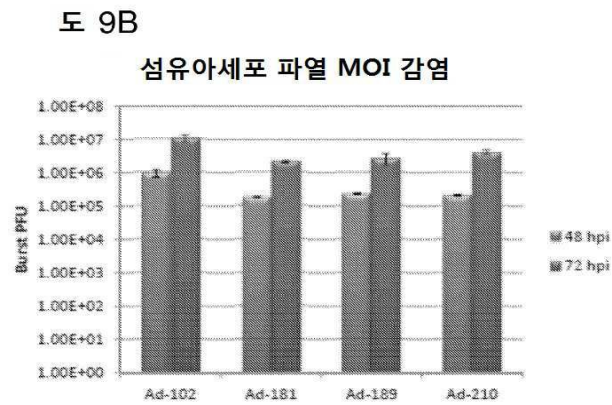
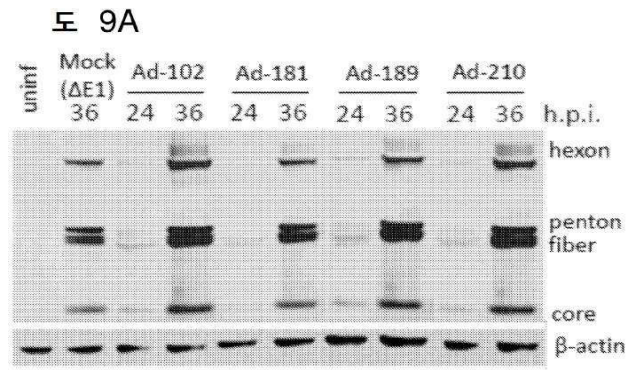
도 8B



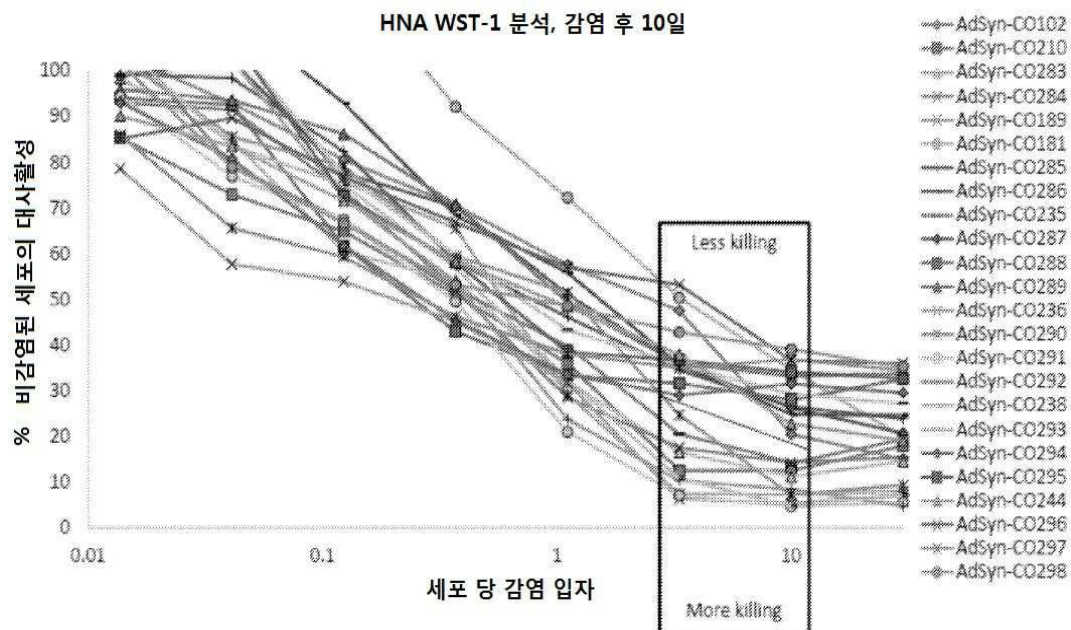
도 8C



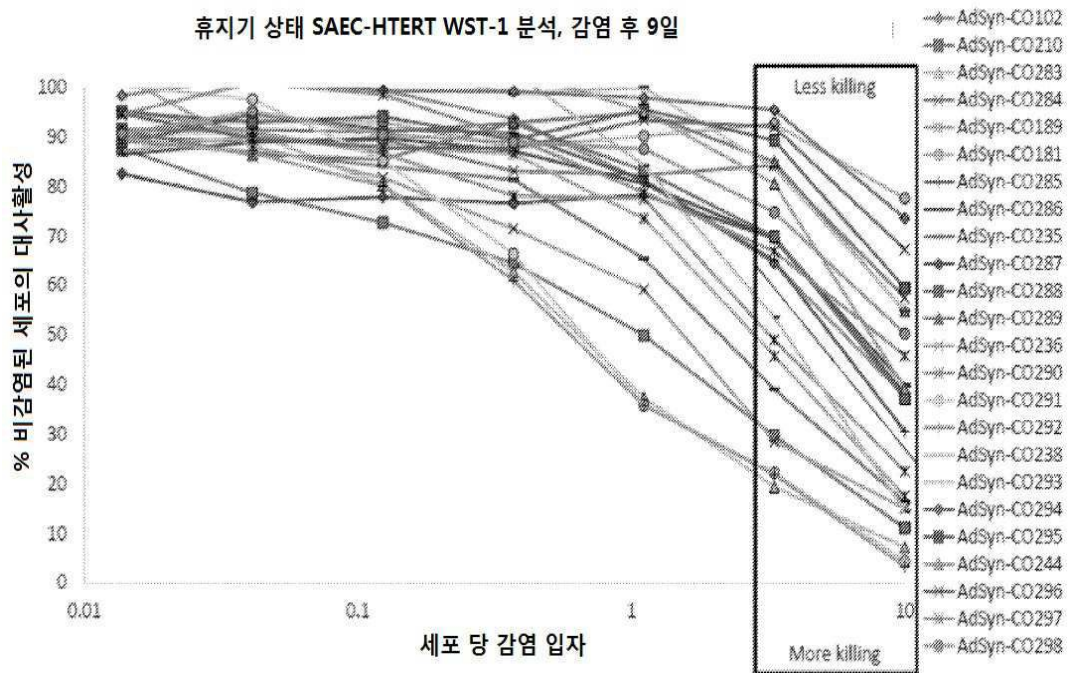
도면9



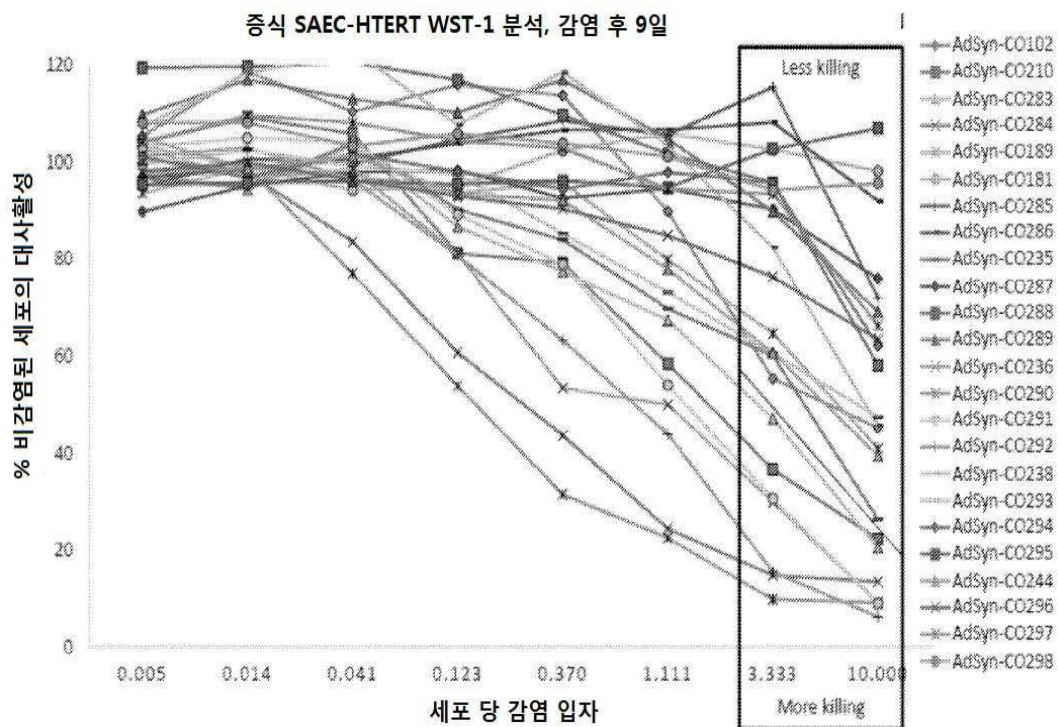
도면10



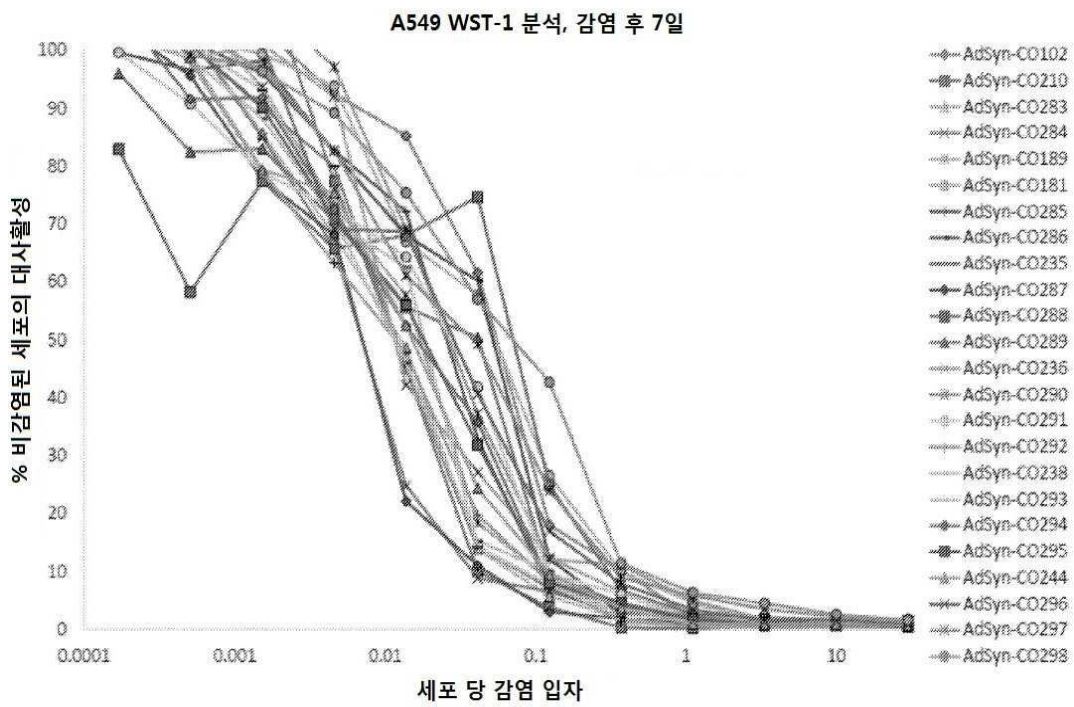
도면11



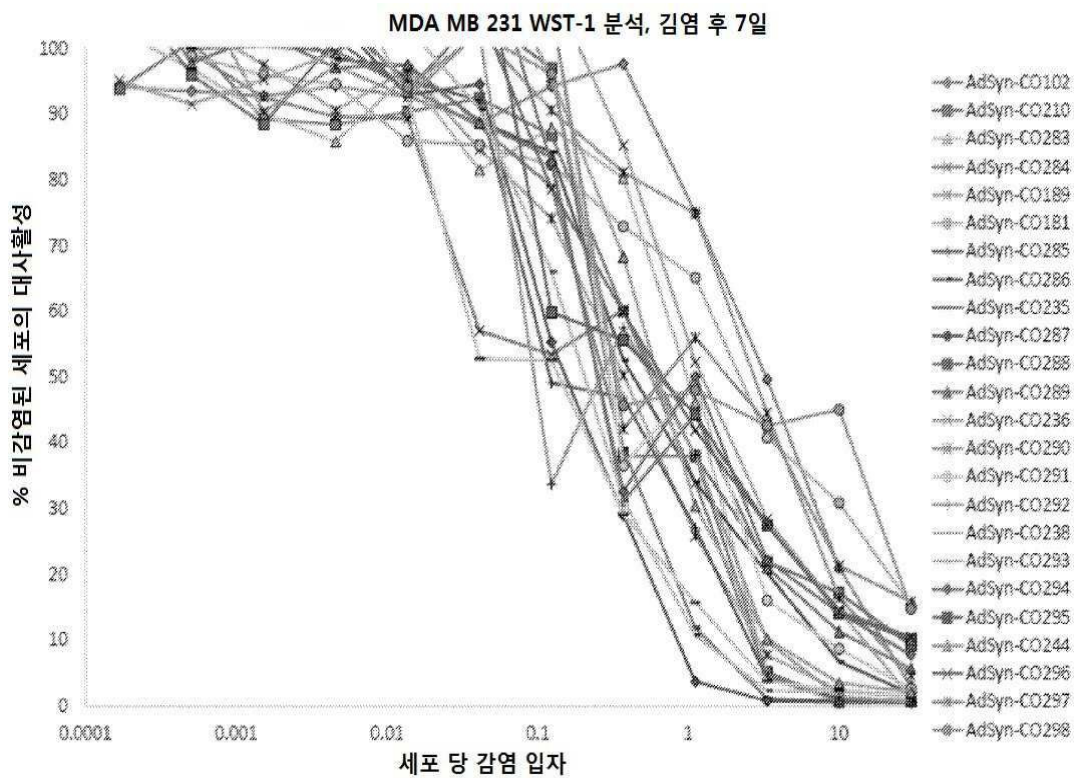
도면12



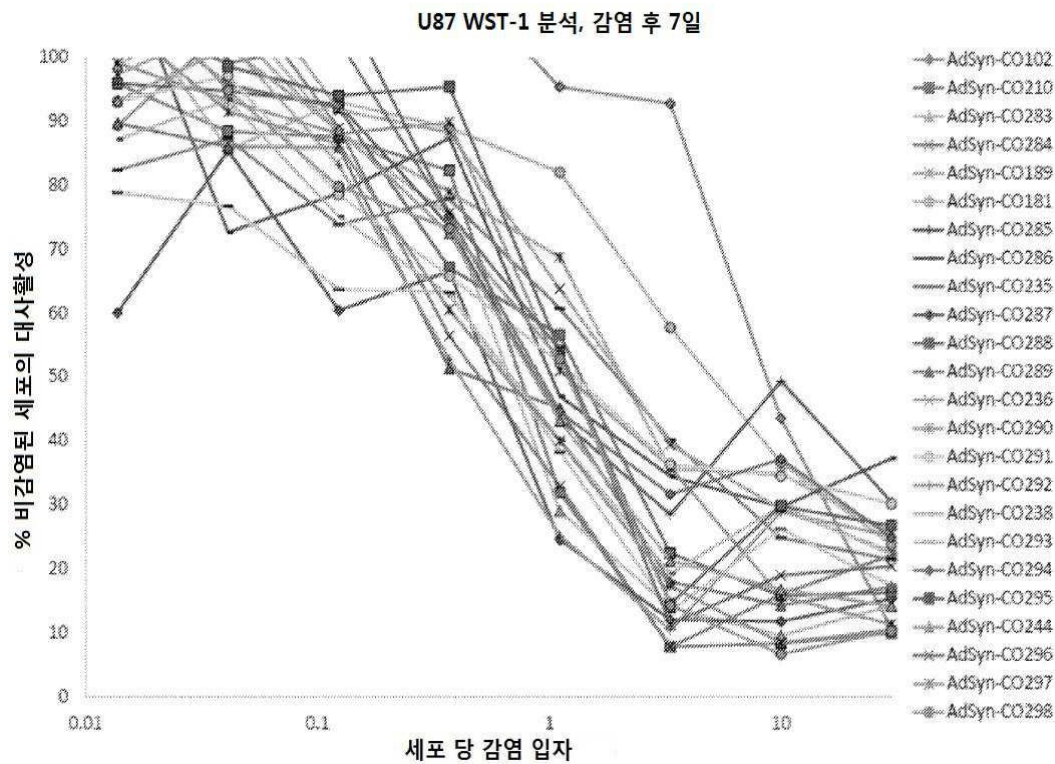
도면13



도면14



도면15



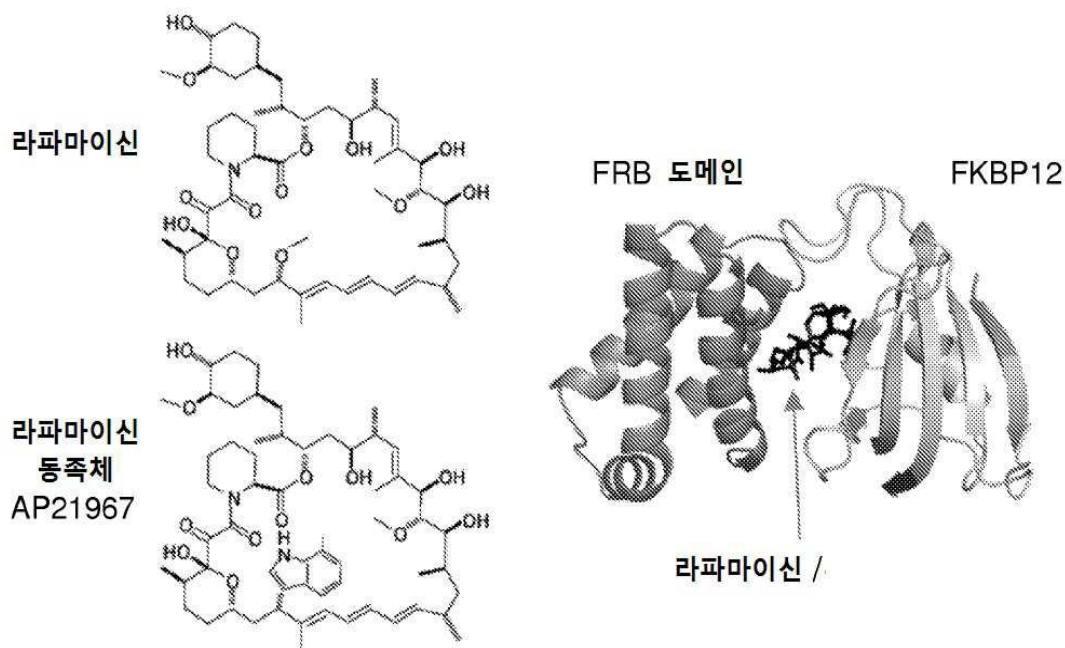
도면16

	NHA			휴지기 상태 SAEC-hTERT			증식 SAEC-hTERT			A549			MDA MB 231			U87		
아데노바이러스, 개시 MOI	10	3.3	1.1	10	3.3	1.1	10	3.3	1.1	10	3.3	1.1	10	3.3	1.1	10	3.3	1.1
AdSyn-CO102	27	31	40	8	14	20	4	6	6	3	3	5	6	8	24	34	23	31
AdSyn-CO210	19	25	32	11	30	50	22	37	59	3	3	4	5	7	30	18	11	16
AdSyn-CO283	11	17	32	7	19	37	21	47	67	1	1	1	1	4	46	10	14	29
AdSyn-CO284	14	18	29	57	84	82	13	15	24	0	1	1	1	5	34	16	8	33
AdSyn-CO236	5	6	32	15	29	59	9	30	50	1	1	1	2	8	26	35	36	69
AdSyn-CO290	5	21	32	23	49	78	9	10	22	3	3	6	13	27	59	9	11	21
AdSyn-CO291	5	7	21	4	22	36	9	31	54	3	2	2	2	4	9	49	28	43
AdSyn-CO292	8	10	24	3	22	36	6	16	44	0	0	1	7	20	34	30	34	47
AdSyn-CO189	28	32	40	17	46	74	41	65	80	3	3	5	3	8	17	23	37	27
AdSyn-CO181	39	46	50	78	93	90	98	103	106	3	3	3	20	27	53	32	31	28
AdSyn-CO285	27	35	46	31	65	81	72	116	106	1	2	3	17	22	38	16	22	57
AdSyn-CO286	25	36	56	40	70	81	92	108	107	1	1	2	11	21	44	14	18	45
AdSyn-CO235	14	21	40	16	39	65	26	60	70	1	1	2	15	28	52	29	39	64
AdSyn-CO287	26	36	51	38	69	78	76	90	95	1	2	5	16	44	75	16	36	51
AdSyn-CO288	28	32	33	37	70	83	58	96	102	1	1	2	9	16	49	34	36	52
AdSyn-CO289	33	36	49	55	85	95	69	90	103	0	0	1	1	10	38	29	10	25
AdSyn-CO238	5	11	33	15	54	84	46	82	103	1	2	2	2	2	11	30	19	40
AdSyn-CO293	29	36	43	55	84	100	47	61	73	1	1	2	2	4	16	26	13	38
AdSyn-CO294	31	29	34	74	95	98	62	96	98	1	1	2	14	22	50	37	32	44
AdSyn-CO295	34	37	38	59	89	96	107	103	95	1	1	2	14	28	44	30	14	54
AdSyn-CO244	23	38	48	39	81	95	39	61	78	1	1	1	3	10	30	17	21	43
AdSyn-CO296	37	53	57	67	92	93	64	76	85	2	1	3	14	27	42	19	11	54
AdSyn-CO297	37	35	38	46	67	79	66	94	95	2	4	6	21	43	56	8	17	40
AdSyn-CO298	39	43	49	50	75	88	96	94	101	3	4	6	45	43	48	7	14	53

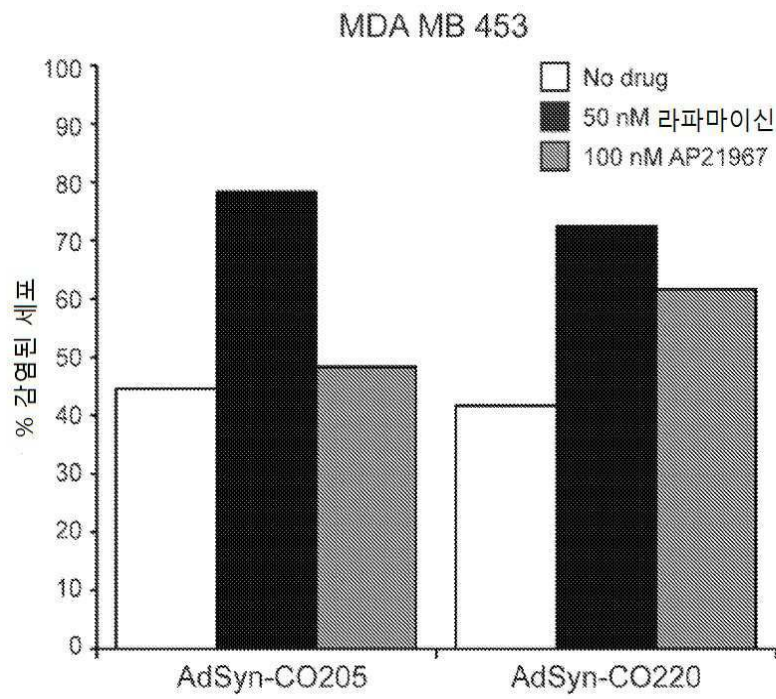
도면17

아데노바이러스, 개시 MOI	U2OS							SaOS2						
	30	10	3.33	1.11	0.37	0.12	0.04	30	10	3.33	1.11	0.37	0.12	0.04
AdSyn-CO102	7	6	5	4	8	19	29	13	52	67	92	105	103	100
AdSyn-CO210	5	4	4	4	10	19	47	8	47	66	97	100	102	100
AdSyn-CO283	7	5	4	5	11	19	49	12	50	73	97	107	106	103
AdSyn-CO284	6	4	3	3	2	3	5	8	11	24	42	68	87	93
AdSyn-CO236	6	5	4	3	3	10	16	11	46	71	96	105	104	99
AdSyn-CO290	5	4	3	3	4	7	27	27	51	60	77	99	98	98
AdSyn-CO291	6	5	4	4	8	17	31	7	16	43	76	96	101	99
AdSyn-CO292	4	3	3	3	4	12	21	11	38	56	78	96	101	100
AdSyn-CO189	7	6	6	7	12	21	32	8	34	69	92	105	106	98
AdSyn-CO181	6	5	4	6	11	19	31	10	40	59	87	104	107	104
AdSyn-CO285	6	5	5	4	6	12	25	8	25	44	65	87	94	99
AdSyn-CO286	5	5	4	5	6	8	18	7	33	58	76	90	96	97
AdSyn-CO235	6	5	5	7	19	31	47	10	60	86	96	106	104	104
AdSyn-CO287	5	4	4	8	18	30	58	5	25	62	88	100	99	99
AdSyn-CO288	8	7	6	6	10	24	44	7	16	44	67	87	93	94
AdSyn-CO289	6	5	4	4	7	18	39	6	13	37	62	83	90	93
AdSyn-CO238	10	9	7	8	16	29	43	8	32	49	65	86	85	89
AdSyn-CO293	7	6	5	5	11	19	39	8	23	34	41	61	87	83
AdSyn-CO294	7	6	5	4	6	11	29	6	8	15	25	42	71	94
AdSyn-CO295	6	6	8	14	23	54	90	34	43	65	82	86	91	97
AdSyn-CO244	7	6	7	13	23	37	52	15	56	73	94	103	103	99
AdSyn-CO296	6	5	5	8	17	29	41	10	33	52	70	85	97	100
AdSyn-CO297	4	4	4	5	11	20	40	4	15	28	48	68	86	97
AdSyn-CO298	4	3	4	5	10	25	40	2	10	22	40	67	80	96

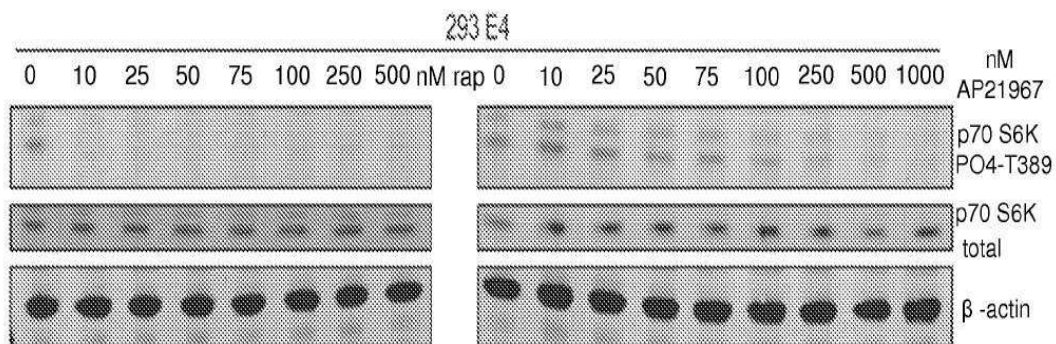
도면18a



도면18b

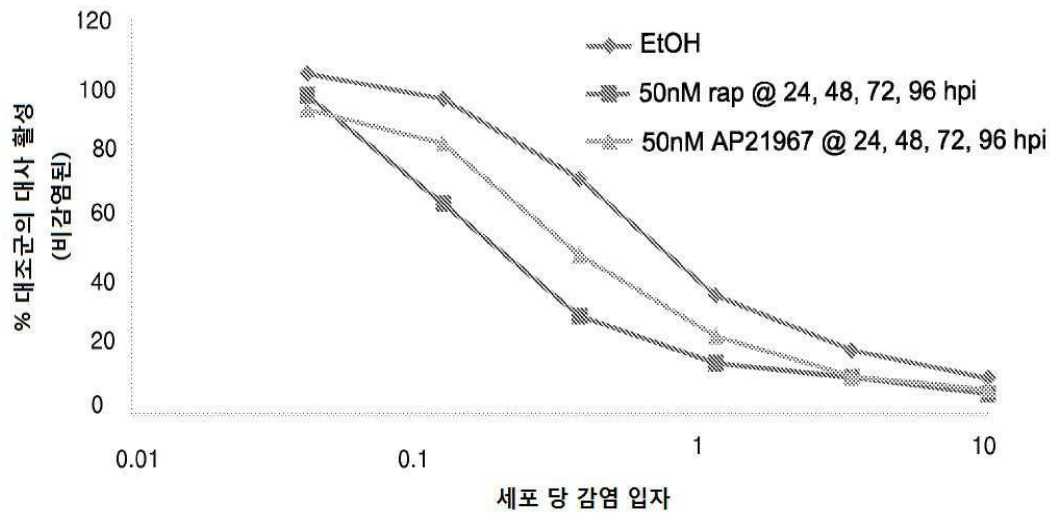


도면18c



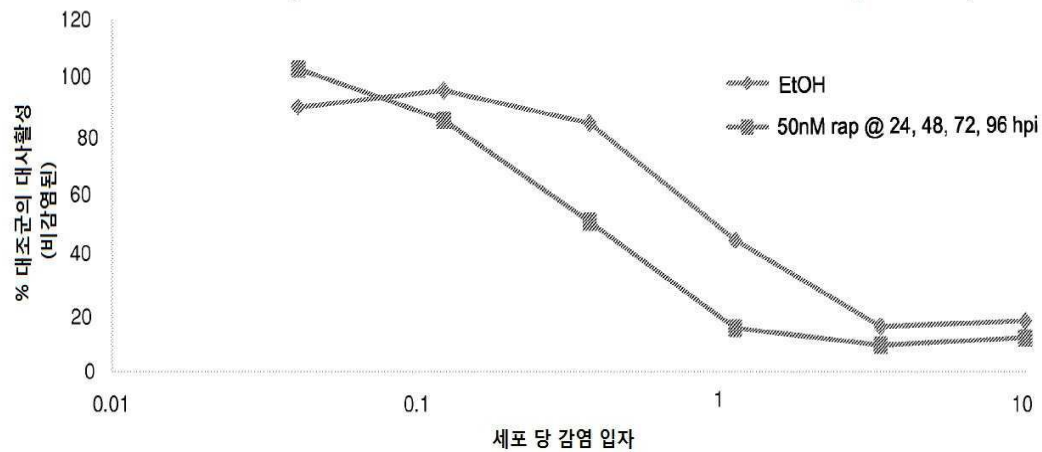
도면18d

AdSyn-CO312 감염된 HS578T의 세포 생존능, 9dpi

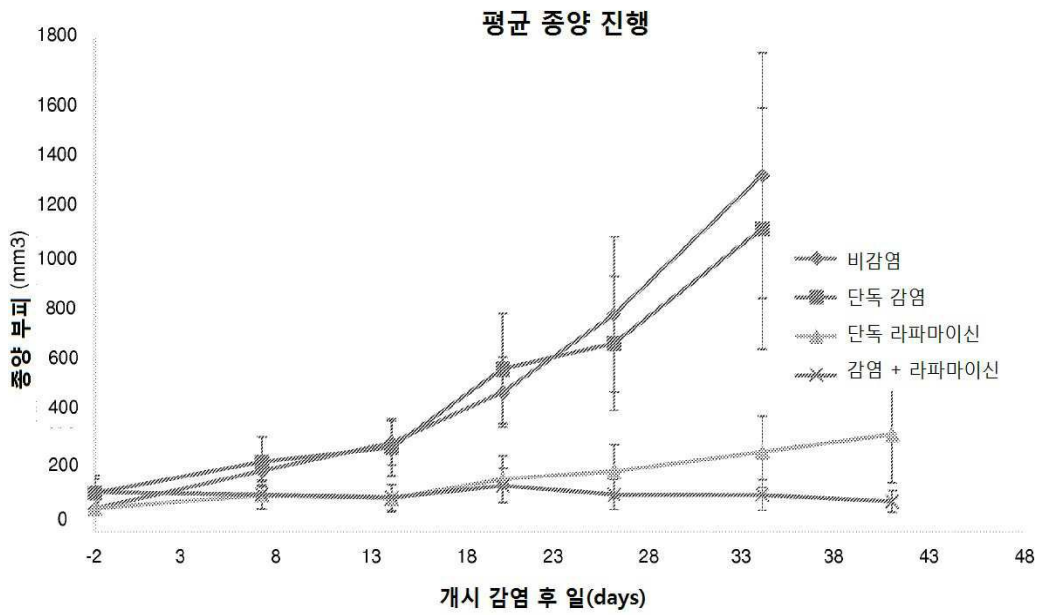


도면18e

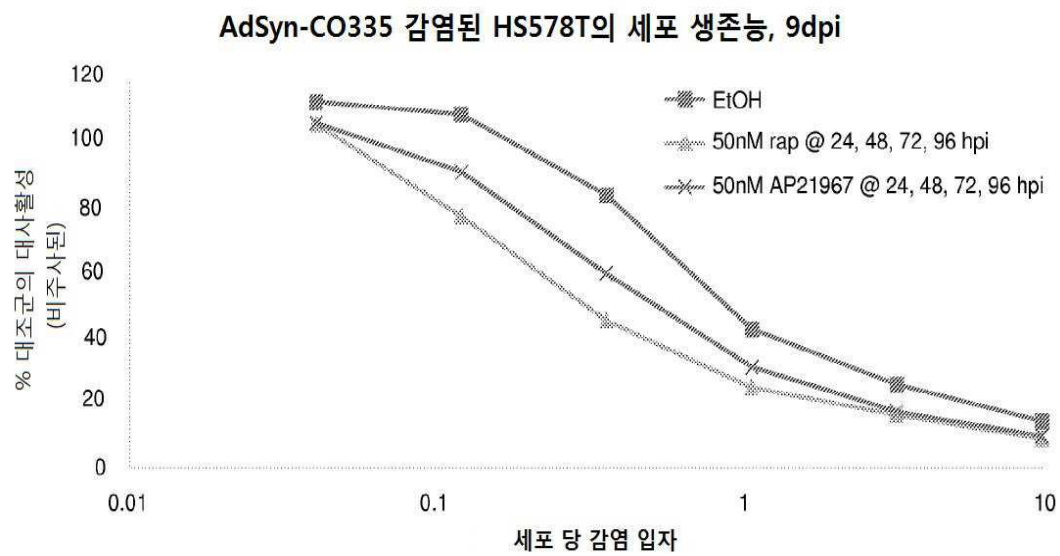
AdSyn-CO313 감염된 HS578T의 세포 생존능, 9dpi



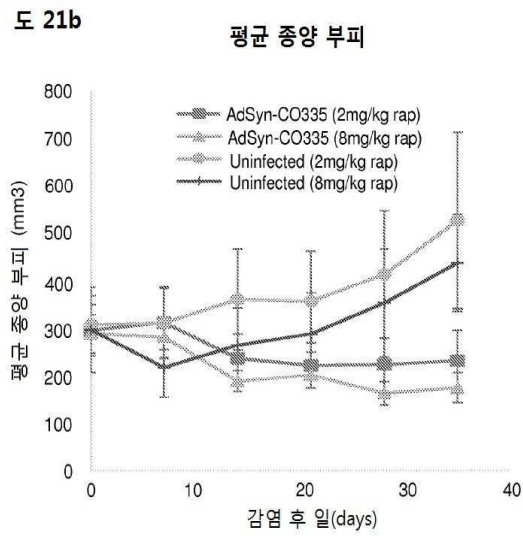
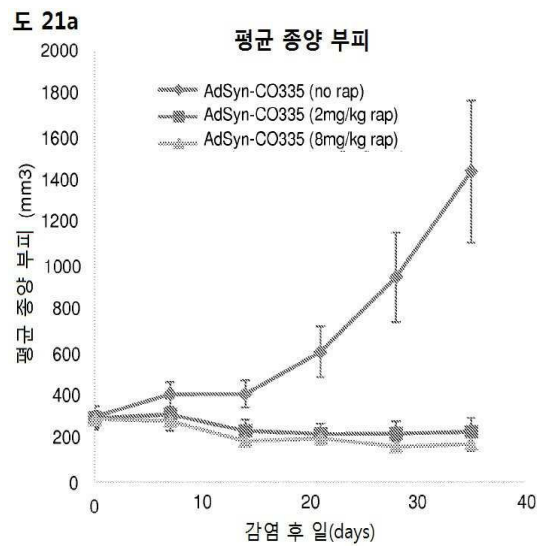
도면19



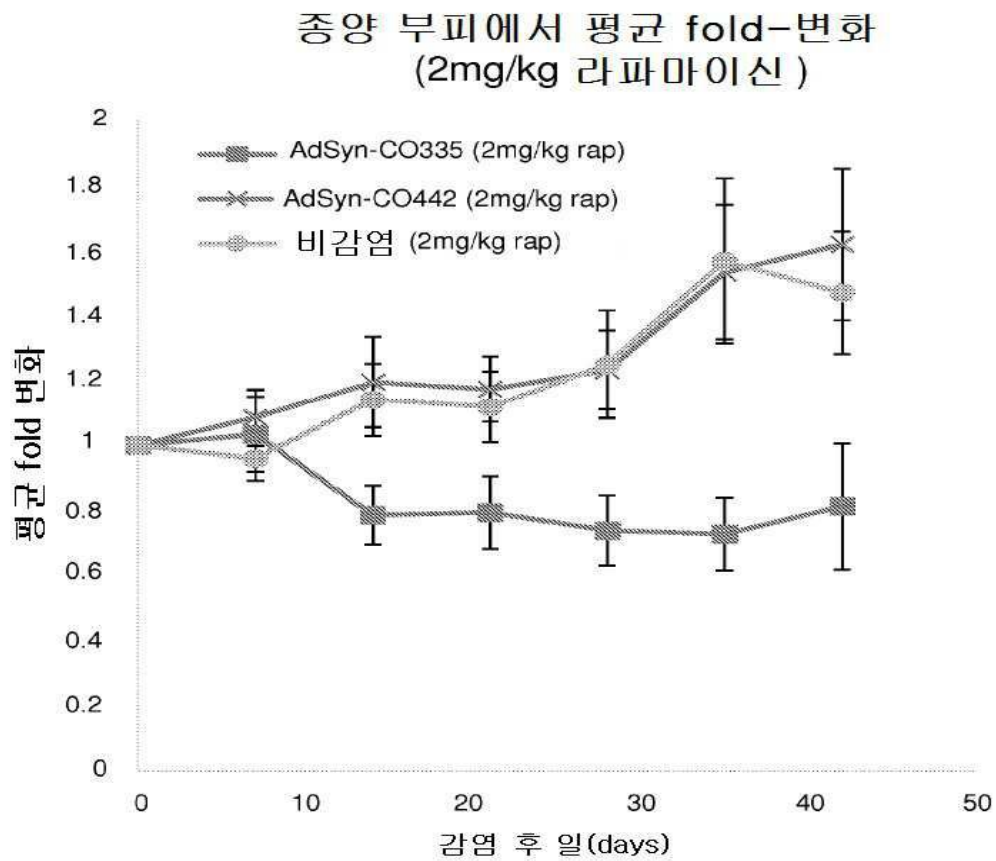
도면20



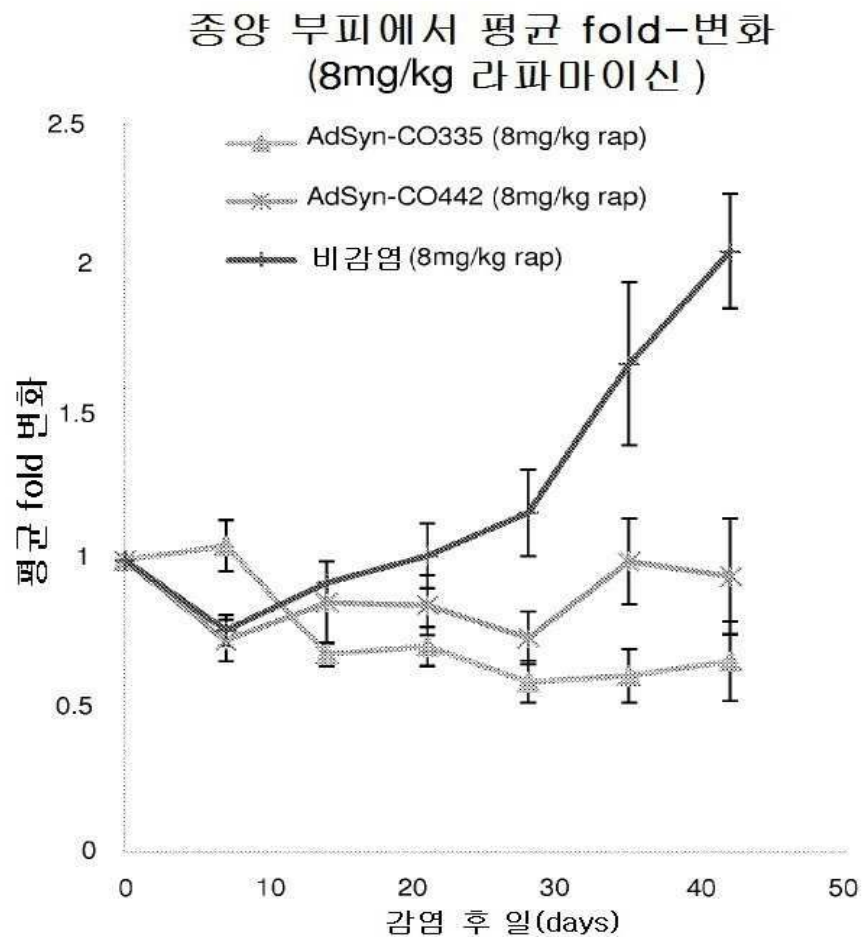
도면21a



도면21c

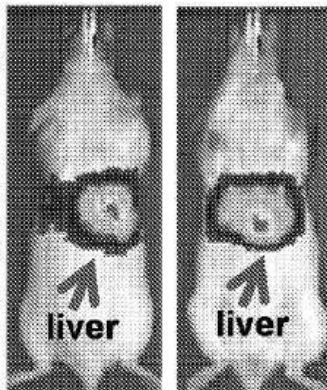


도면21d



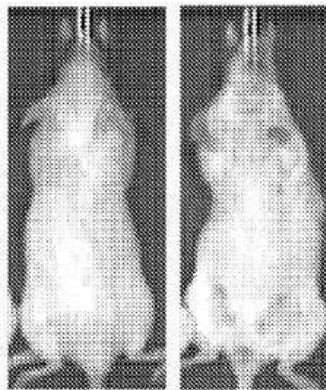
도면22

도 22A



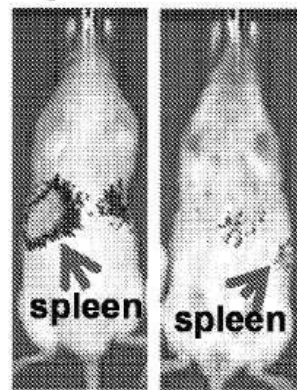
AdSyn-CO199
E1 모듈 - ΔE1+
 EF1α-루시페라제

도 22B



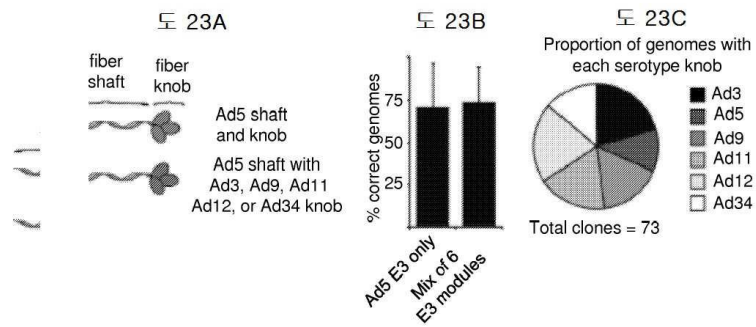
AdSyn-CO200
E1 모듈 - ΔE1+
 EF1α-루시페라제
 (+miR122 결합)

도 22C

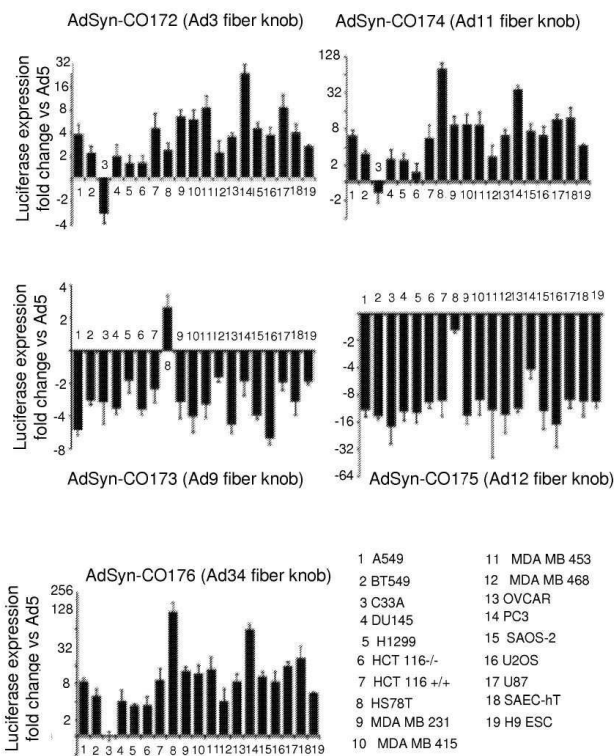


AdSyn-CO171
E1 모듈 - ΔE1+
 EF1α-루시페라제
 (+miR122 결합)
 코어 모듈 -
 핵손 E451Q

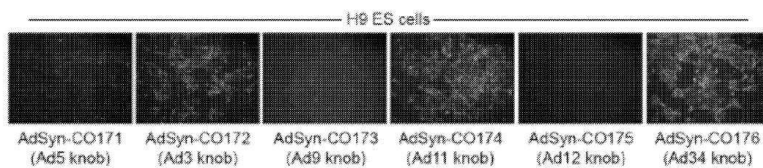
도면23



도 23D

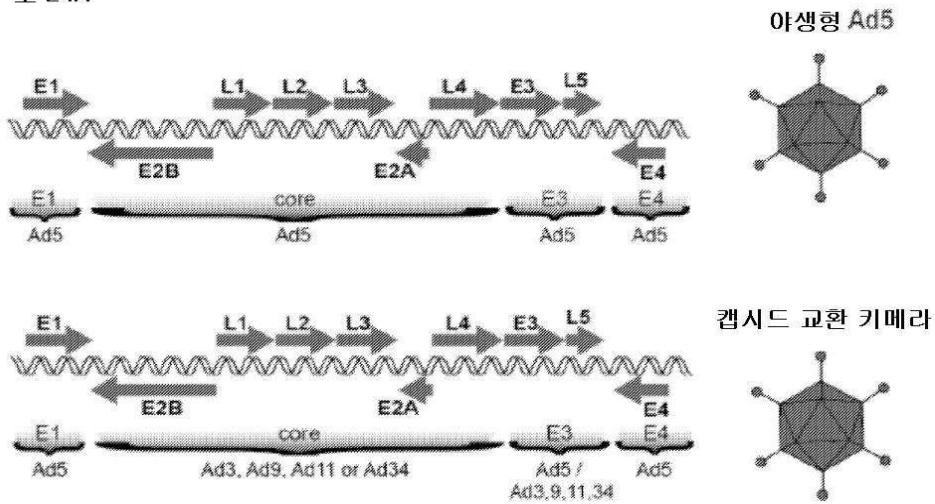


도 23E



도면24

도 24A

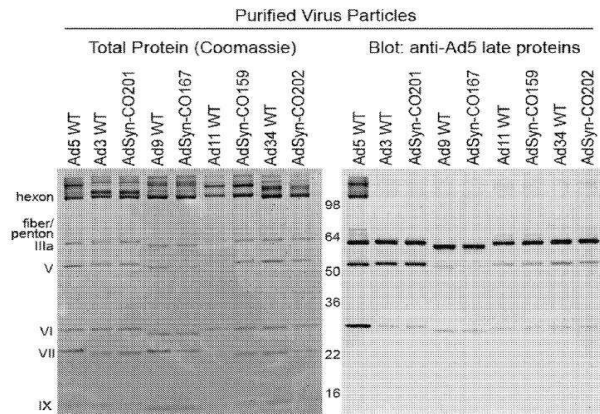


도 24B

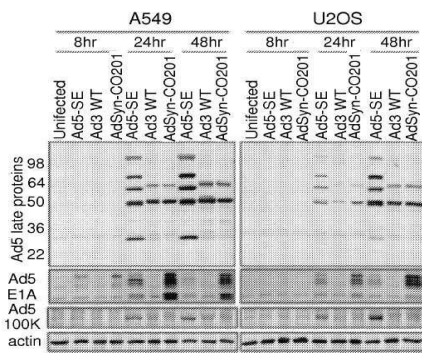
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		E1	core	E3	E4	
AdSyn-CQ150	AdSLIC	Ad11 pIX	All Ad11	Ad11 Uexon and fiber	None	Yes
AdSyn-CQ167	AdSLIC	Ad9 pIX	All Ad9	Ad9 Uexon and fiber	None	Yes
AdSyn-CQ168	AdSLIC	Ad12 pIX	All Ad12	Ad12 Uexon and fiber	None	No
AdSyn-CQ201	AdSLIC	Ad3 pIX	All Ad3	Ad3 Uexon and fiber	None	Yes
AdSyn-CQ202	AdSLIC	Ad34 pIX	All Ad34	Ad34 Uexon and fiber	None	Yes
AdSyn-CQ185	AdSLIC	MAV-1 pIX, ΔE1-CMV-GFP	All MAV-1	MAV-1 Uexon and fiber	None	No
AdSyn-CQ254	AdSLIC	Ad12 ITR/Ψ, Ad12 pIX	All Ad12	Ad12 Uexon and fiber	Ad12 ITR	No
AdSyn-CQ255	AdSLIC	MAV-1 pIX, MAV-1 ITR/Ψ, ΔE1-CMV-GFP	All MAV-1	MAV-1 Uexon and fiber	MAV-1 ITR	No

도면25

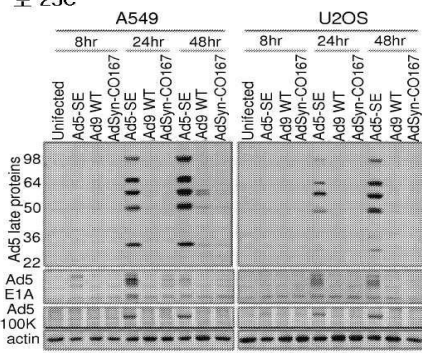
도 25A



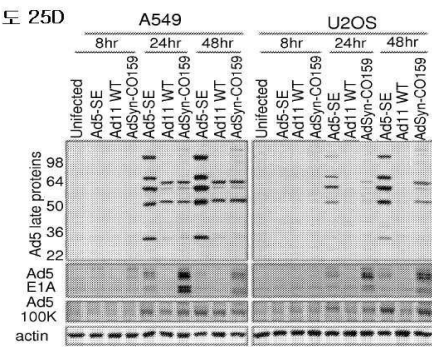
도 25B



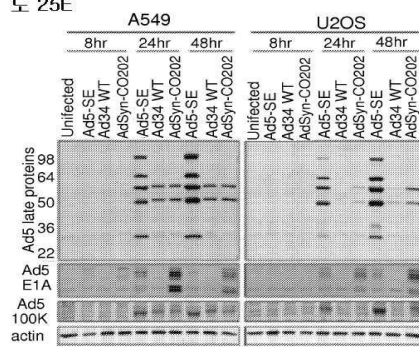
도 25C



도 25D

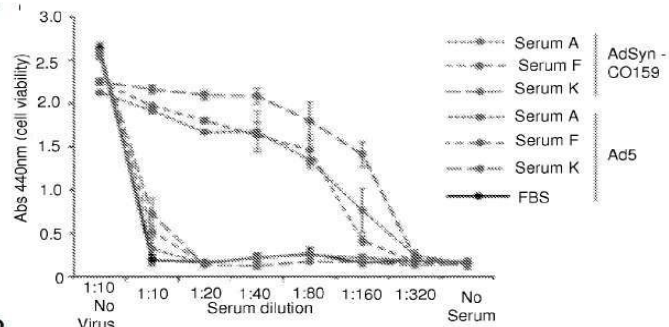


도 25E

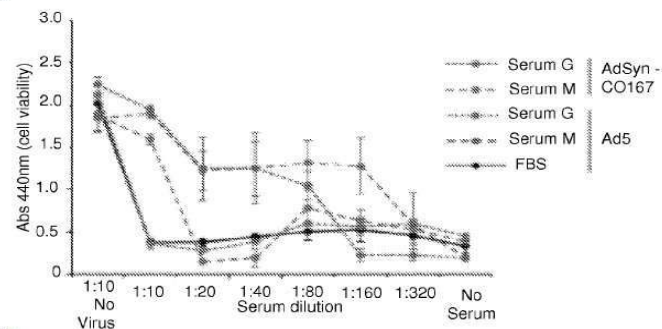


도면26

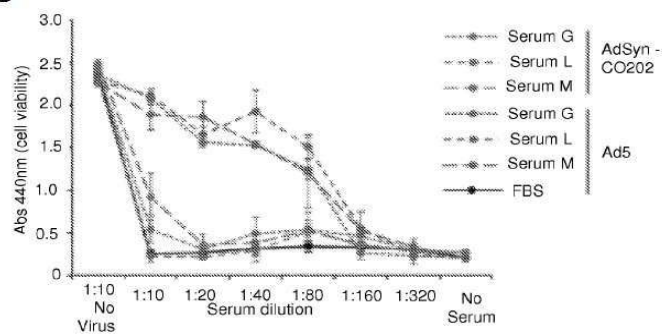
도 26A



도 26B



도 26C



도면27

[illegible]

도면28

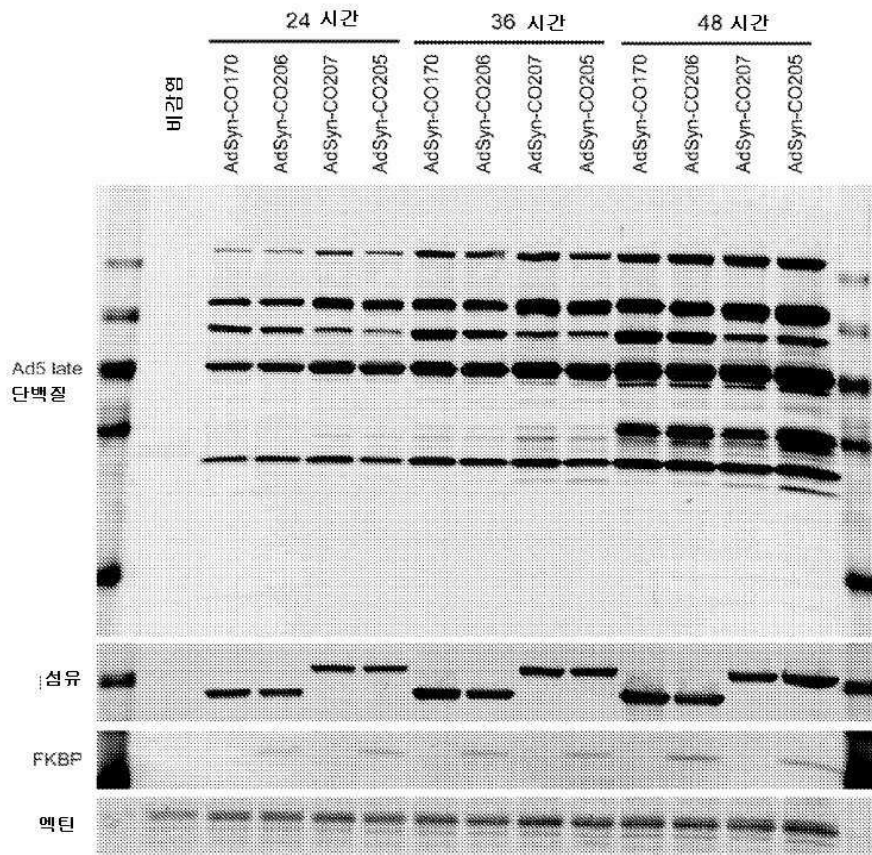
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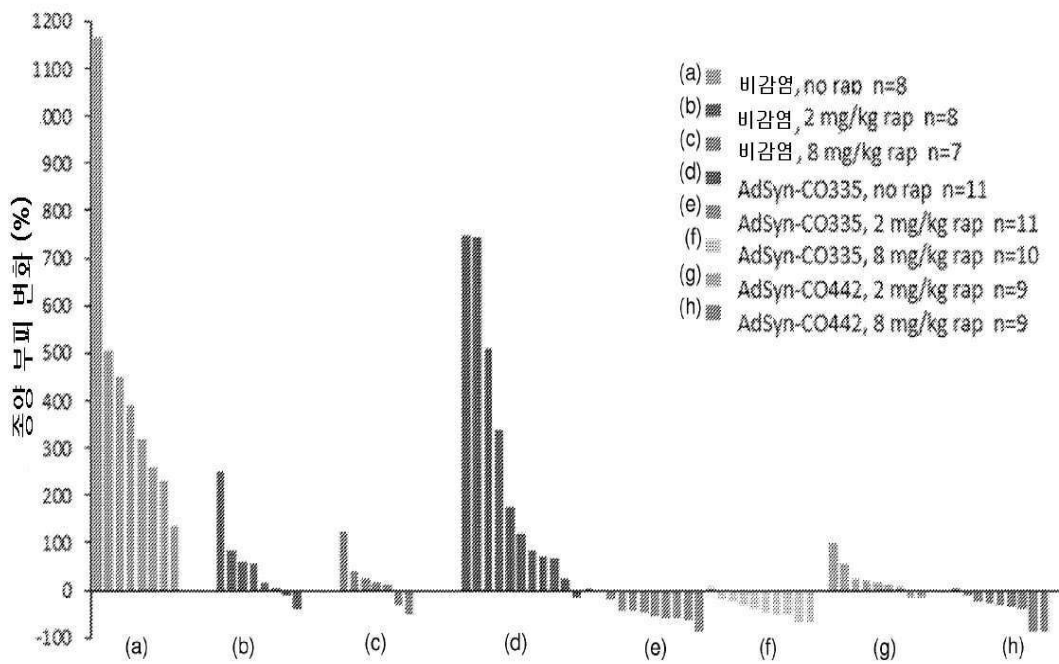
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Species_G_Ad52_E4orf1            MAD--QHIYVHLLGRR AFLPQQQGYSNMYVLFSPEDFVLAPRGIILSLQ
*      : : * :      . * * :      * : : * * : * * : * * :
51                                     100
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Species_B_Ad7_E4orf1             LSI IIPRGYVGCFFSLTDANMSGLYASSRI IHAGHREELSVLLFNHDDR
Species_C_Ad2_E4orf1             LRVCIIPPGYFGRFLALTDVNQPDVFTESYIMTPDMTEELSVVLFNHGDQF
Species_C_Ad5_E4orf1             LRVCIIPPGYFGRFLALTDVNQPDVFTESYIMTPDMTEELSVVLFNHGDQF
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Species_E_Ad4_E4orf1             LSIVVPQGFIGRFFSLTDANVPGVYASSRI IHAGHREGLSVMLFNHNVSF
Species_G_Ad52_E4orf1            LSLDIPTGYLGRFFSVADMNVRGVLLCAQEIQPSTWWEVSVVLFNHSDEF
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101                                     128
Species_A_Ad12_predicted_E4orf1  CDVRKQPVARLLLSRVVFPVCQASLI
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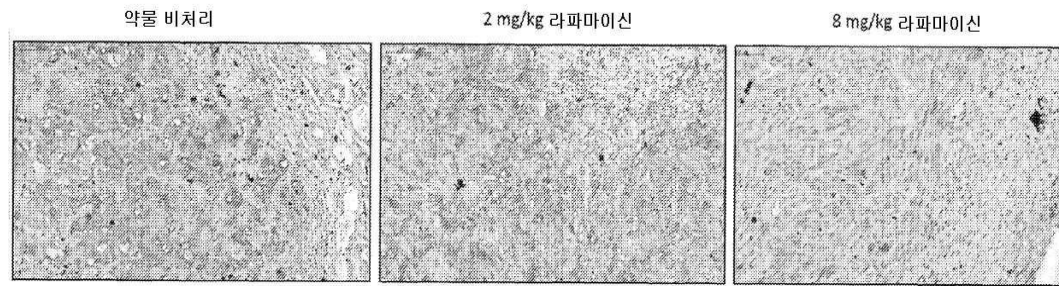

도면29



도면30



도면31



서열 목록

SEQUENCE LISTING

<110> SALK INSTITUTE FOR BIOLOGICAL STUDIES

<120> ONCOLYTIC TUMOR VIRUSES AND METHODS OF USE

<130> 7158-93694-02

<150> US 62/054,724

<151> 2014-09-24

<160> 98

<170> PatentIn version 3.5

<210> 1

<211> 35941

<212> DNA

<213> Artificial Sequence

<220><223> Recombinant adenovirus genome (AdSyn-C0102)

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 aaataacaaa aaaacattta aacattagaa gcctgtctta caacaggaaa aacaaccctt 35160
 ataagcataa gacggactac ggccatgccg gcgtgaccgt aaaaaaactg gtcacgtga 35220
 ttaaaaagca ccaccgacag ctctcgggtc atgtccggag tcataatgta agactcggta 35280

aacacatcag gttgattcac atcggtcagt gctaaaaagc gaccgaaata gcccggggga 35340
 atacataccc gcaggcgtag agacaacatt acagcccca taggaggtat aacaaaatta 35400
 ataggagaga aaaacacata aacacctgaa aaacctcct gcctaggcaa aatagcacc 35460
 tcccgtcca gaacaacata cagcgttcc acagcggcag ccataacagt cagccttacc 35520
 agtaaaaaag aaaacctatt aaaaaaacac cactcgacac ggcaccagct caatcagtca 35580
 cagtgtaaaa aaggccaag tgcagagcga gtatatatag gactaaaaaa tgacgtaacg 35640
 gttaaagtcc acaaaaaaca ccagaaaaac cgcacgcgaa cctacgccca gaaacgaaag 35700

ccaaaaaacc cacaacttcc tcaaatcgtc acttccgttt tcccacgtta cgtcacttcc 35760
 cattttaaga aaactacaat tccaacaca tacaagtac tccgcctaa aacctacgtc 35820
 acccgccccg ttcccacgcc ccgcgccacg tcacaaactc caccctca ttatcatatt 35880
 ggcttcaatc caaataagg tatattattg atgatg 35916

<210> 32

<211> 289

<212> PRT

<213> Human adenovirus type 5

<400> 32

Met Arg His Ile Ile Cys His Gly Gly Val Ile Thr Glu Glu Met Ala

1 5 10 15

Ala Ser Leu Leu Asp Gln Leu Ile Glu Glu Val Leu Ala Asp Asn Leu

20 25 30

Pro Pro Pro Ser His Phe Glu Pro Pro Thr Leu His Glu Leu Tyr Asp

35 40 45

Leu Asp Val Thr Ala Pro Glu Asp Pro Asn Glu Glu Ala Val Ser Gln

50 55 60

Ile Phe Pro Asp Ser Val Met Leu Ala Val Gln Glu Gly Ile Asp Leu

65					70					75					80
Leu	Thr	Phe	Pro	Pro	Ala	Pro	Gly	Ser	Pro	Glu	Pro	Pro	His	Leu	Ser
				85						90					
Arg	Gln	Pro	Glu	Gln	Pro	Glu	Gln	Arg	Ala	Leu	Gly	Pro	Val	Ser	Met
				100						105					
Pro	Asn	Leu	Val	Pro	Glu	Val	Ile	Asp	Leu	Thr	Cys	His	Glu	Ala	Gly
				115						120					
Phe	Pro	Pro	Ser	Asp	Asp	Glu	Asp	Glu	Glu	Gly	Glu	Glu	Phe	Val	Leu
				130						135					
										140					
Asp	Tyr	Val	Glu	His	Pro	Gly	His	Gly	Cys	Arg	Ser	Cys	His	Tyr	His
145				150				155				160			
Arg	Arg	Asn	Thr	Gly	Asp	Pro	Asp	Ile	Met	Cys	Ser	Leu	Cys	Tyr	Met
				165						170					
Arg	Thr	Cys	Gly	Met	Phe	Val	Tyr	Ser	Pro	Val	Ser	Glu	Pro	Glu	Pro
				180						185					
										190					
Glu	Pro	Glu	Pro	Glu	Pro	Glu	Pro	Ala	Arg	Pro	Thr	Arg	Arg	Pro	Lys
				195						200					
										205					
Met	Ala	Pro	Ala	Ile	Leu	Arg	Arg	Pro	Thr	Ser	Pro	Val	Ser	Arg	Glu
210				215				220							
Cys	Asn	Ser	Ser	Thr	Asp	Ser	Cys	Asp	Ser	Gly	Pro	Ser	Asn	Thr	Pro
225				230				235				240			
Pro	Glu	Ile	His	Pro	Val	Val	Pro	Leu	Cys	Pro	Ile	Lys	Pro	Val	Ala
				245						250					
										255					
Val	Arg	Val	Gly	Gly	Arg	Arg	Gln	Ala	Val	Glu	Cys	Ile	Glu	Asp	Leu
				260						265					
										270					
Leu	Asn	Glu	Pro	Gly	Gln	Pro	Leu	Asp	Leu	Ser	Cys	Lys	Arg	Pro	Arg
275				280				285							
Pro															

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic polypeptide

<400> 33

Met Arg His Ile Ile Cys His Gly Gly Val Ile Thr Glu Glu Met Ala

1 5 10 15

Ala Ser Leu Leu Asp Gln Leu Ile Glu Glu Val Leu Ala Asp Asn Leu

20 25 30

Pro Pro Pro Ser His Phe Glu Pro Pro Thr Leu His Glu Leu Tyr Asp

35 40 45

Leu Asp Val Thr Ala Pro Glu Asp Pro Asn Glu Glu Ala Val Ser Gln

50 55 60

Ile Phe Pro Asp Ser Val Met Leu Ala Val Gln Glu Gly Ile Asp Leu

65 70 75 80

Leu Thr Phe Pro Pro Ala Pro Gly Ser Pro Glu Pro Pro His Leu Ser

85 90 95

Arg Gln Pro Glu Gln Pro Glu Gln Arg Ala Leu Gly Pro Val Ser Met

100 105 110

Pro Asn Leu Val Pro Glu Val Ile Asp Ala Gly Phe Pro Pro Ser Asp

115 120 125

Asp Glu Asp Glu Glu Gly Glu Glu Phe Val Leu Asp Tyr Val Glu His

130 135 140

Pro Gly His Gly Cys Arg Ser Cys His Tyr His Arg Arg Asn Thr Gly

145 150 155 160

Asp Pro Asp Ile Met Cys Ser Leu Cys Tyr Met Arg Thr Cys Gly Met

165 170 175

Phe Val Tyr Ser Pro Val Ser Glu Pro Glu Pro Glu Pro Glu Pro Glu

180 185 190

Pro Glu Pro Ala Arg Pro Thr Arg Arg Pro Lys Met Ala Pro Ala Ile

195 200 205

Leu Arg Arg Pro Thr Ser Pro Val Ser Arg Glu Cys Asn Ser Ser Thr

210 215 220

Asp Ser Cys Asp Ser Gly Pro Ser Asn Thr Pro Pro Glu Ile His Pro

225 230 235 240

Val Val Pro Leu Cys Pro Ile Lys Pro Val Ala Val Arg Val Gly Gly

245 250 255

Arg Arg Gln Ala Val Glu Cys Ile Glu Asp Leu Leu Asn Glu Pro Gly

260 265 270

Gln Pro Leu Asp Leu Ser Cys Lys Arg Pro Arg Pro

275 280

<210> 34

<211

> 289

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic polypeptide

<400> 34

Met Arg His Ile Ile Cys His Gly Gly Val Ile Thr Glu Glu Met Ala

1 5 10 15

Ala Ser Leu Leu Asp Gln Leu Ile Glu Glu Val Leu Ala Asp Asn Leu

20 25 30

Pro Pro Pro Ser His Phe Glu Pro Pro Thr Leu His Glu Leu Tyr Asp

35 40 45

Leu Asp Val Thr Ala Pro Glu Asp Pro Asn Glu Glu Ala Val Ser Gln

50 55 60

Ile Phe Pro Asp Ser Val Met Leu Ala Val Gln Glu Gly Ile Asp Leu

65 70 75 80

Leu Thr Phe Pro Pro Ala Pro Gly Ser Pro Glu Pro Pro His Leu Ser

85 90 95

Arg Gln Pro Glu Gln Pro Glu Gln Arg Ala Leu Gly Pro Val Ser Met

100 105 110

Pro Asn Leu Val Pro Glu Val Ile Asp Leu Thr Gly His Glu Ala Gly

115 120 125

Phe Pro Pro Ser Asp Asp Glu Asp Glu Glu Gly Glu Glu Phe Val Leu
130 135 140
Asp Tyr Val Glu His Pro Gly His Gly Cys Arg Ser Cys His Tyr His
145 150 155 160
Arg Arg Asn Thr Gly Asp Pro Asp Ile Met Cys Ser Leu Cys Tyr Met
165 170 175
Arg Thr Cys Gly Met Phe Val Tyr Ser Pro Val Ser Glu Pro Glu Pro
180 185 190
Glu Pro Glu Pro Glu Pro Glu Pro Ala Arg Pro Thr Arg Arg Pro Lys
195 200 205
Met Ala Pro Ala Ile Leu Arg Arg Pro Thr Ser Pro Val Ser Arg Glu
210 215 220
Cys Asn Ser Ser Thr Asp Ser Cys Asp Ser Gly Pro Ser Asn Thr Pro
225 230 235 240
Pro Glu Ile His Pro Val Val Pro Leu Cys Pro Ile Lys Pro Val Ala
245 250 255
Val Arg Val Gly Gly Arg Arg Gln Ala Val Glu Cys Ile Glu Asp Leu
260 265 270
Leu Asn Glu Pro Gly Gln Pro Leu Asp Leu Ser Cys Lys Arg Pro Arg
275 280 285
Pro

<210> 35

<211> 278

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic polypeptide

<400> 35

Met Glu Glu Met Ala Ala Ser Leu Leu Asp Gln Leu Ile Glu Glu Val

1 5 10 15

Leu Ala Asp Asn Leu Pro Pro Pro Ser His Phe Glu Pro Pro Thr Leu

20 25 30

His Glu Leu Tyr Asp Leu Asp Val Thr Ala Pro Glu Asp Pro Asn Glu
 35 40 45
 Glu Ala Val Ser Gln Ile Phe Pro Asp Ser Val Met Leu Ala Val Gln
 50 55 60
 Glu Gly Ile Asp Leu Leu Thr Phe Pro Pro Ala Pro Gly Ser Pro Glu
 65 70 75 80
 Pro Pro His Leu Ser Arg Gln Pro Glu Gln Pro Glu Gln Arg Ala Leu
 85 90 95
 Gly Pro Val Ser Met Pro Asn Leu Val Pro Glu Val Ile Asp Leu Thr
 100 105 110
 Cys His Glu Ala Gly Phe Pro Pro Ser Asp Asp Glu Asp Glu Glu Gly
 115 120 125
 Glu Glu Phe Val Leu Asp Tyr Val Glu His Pro Gly His Gly Cys Arg
 130 135 140
 Ser Cys His Tyr His Arg Arg Asn Thr Gly Asp Pro Asp Ile Met Cys
 145 150 155 160
 Ser Leu Cys Tyr Met Arg Thr Cys Gly Met Phe Val Tyr Ser Pro Val
 165 170 175
 Ser Glu Pro Glu Pro Glu Pro Glu Pro Glu Pro Glu Pro Ala Arg Pro
 180 185 190
 Thr Arg Arg Pro Lys Met Ala Pro Ala Ile Leu Arg Arg Pro Thr Ser
 195 200 205
 Pro Val Ser Arg Glu Cys Asn Ser Ser Thr Asp Ser Cys Asp Ser Gly
 210 215 220
 Pro Ser Asn Thr Pro Pro Glu Ile His Pro Val Val Pro Leu Cys Pro
 225 230 235 240
 Ile Lys Pro Val Ala Val Arg Val Gly Gly Arg Arg Gln Ala Val Glu
 245 250 255
 Cys Ile Glu Asp Leu Leu Asn Glu Pro Gly Gln Pro Leu Asp Leu Ser
 260 265 270
 Cys Lys Arg Pro Arg Pro

275

<210> 36

<211> 289

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic polypeptide

<400> 36

Met Arg His Ile Ile Cys His Gly Gly Val Ile Thr Glu Glu Met Ala

1 5 10 15

Ala Ser Leu Leu Asp Gln Leu Ile Glu Glu Val Leu Ala Asp Asn Leu

20 25 30

Pro Pro Pro Ser His Phe Glu Pro Pro Thr Leu His Glu Leu His Asp

35 40 45

Leu Asp Val Thr Ala Pro Glu Asp Pro Asn Glu Glu Ala Val Ser Gln

50 55 60

Ile Phe Pro Asp Ser Val Met Leu Ala Val Gln Glu Gly Ile Asp Leu

65 70 75 80

Leu Thr Phe Pro Pro Ala Pro Gly Ser Pro Glu Pro Pro His Leu Ser

85 90 95

Arg Gln Pro Glu Gln Pro Glu Gln Arg Ala Leu Gly Pro Val Ser Met

100 105 110

Pro Asn Leu Val Pro Glu Val Ile Asp Leu Thr Gly His Glu Ala Gly

115 120 125

Phe Pro Pro Ser Asp Asp Glu Asp Glu Glu Gly Glu Glu Phe Val Leu

130 135 140

Asp Tyr Val Glu His Pro Gly His Gly Cys Arg Ser Cys His Tyr His

145 150 155 160

Arg Arg Asn Thr Gly Asp Pro Asp Ile Met Cys Ser Leu Cys Tyr Met

165 170 175

Arg Thr Cys Gly Met Phe Val Tyr Ser Pro Val Ser Glu Pro Glu Pro

180 185 190

Glu Pro Glu Pro Glu Pro Glu Pro Ala Arg Pro Thr Arg Arg Pro Lys

195 200 205
Met Ala Pro Ala Ile Leu Arg Arg Pro Thr Ser Pro Val Ser Arg Glu
210 215 220
Cys Asn Ser Ser Thr Asp Ser Cys Asp Ser Gly Pro Ser Asn Thr Pro

225 230 235 240
Pro Glu Ile His Pro Val Val Pro Leu Cys Pro Ile Lys Pro Val Ala
245 250 255
Val Arg Val Gly Gly Arg Arg Gln Ala Val Glu Cys Ile Glu Asp Leu
260 265 270
Leu Asn Glu Pro Gly Gln Pro Leu Asp Leu Ser Cys Lys Arg Pro Arg
275 280 285
Pro

<210> 37

<211> 278

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic polypeptide

<400> 37

Met Glu Glu Met Ala Ala Ser Leu Leu Asp Gln Leu Ile Glu Glu Val
1 5 10 15
Leu Ala Asp Asn Leu Pro Pro Pro Ser His Phe Glu Pro Pro Thr Leu
20 25 30
His Glu Leu His Asp Leu Asp Val Thr Ala Pro Glu Asp Pro Asn Glu
35 40 45
Glu Ala Val Ser Gln Ile Phe Pro Asp Ser Val Met Leu Ala Val Gln

50 55 60
Glu Gly Ile Asp Leu Leu Thr Phe Pro Pro Ala Pro Gly Ser Pro Glu
65 70 75 80
Pro Pro His Leu Ser Arg Gln Pro Glu Gln Pro Glu Gln Arg Ala Leu
85 90 95
Gly Pro Val Ser Met Pro Asn Leu Val Pro Glu Val Ile Asp Leu Thr

100 105 110
Gly His Glu Ala Gly Phe Pro Pro Ser Asp Asp Glu Asp Glu Glu Gly

115 120 125
Glu Glu Phe Val Leu Asp Tyr Val Glu His Pro Gly His Gly Cys Arg

130 135 140
Ser Cys His Tyr His Arg Arg Asn Thr Gly Asp Pro Asp Ile Met Cys

145 150 155 160
Ser Leu Cys Tyr Met Arg Thr Cys Gly Met Phe Val Tyr Ser Pro Val

165 170 175
Ser Glu Pro Glu Pro Glu Pro Glu Pro Glu Pro Glu Pro Ala Arg Pro

180 185 190
Thr Arg Arg Pro Lys Met Ala Pro Ala Ile Leu Arg Arg Pro Thr Ser

195 200 205
Pro Val Ser Arg Glu Cys Asn Ser Ser Thr Asp Ser Cys Asp Ser Gly

210 215 220
Pro Ser Asn Thr Pro Pro Glu Ile His Pro Val Val Pro Leu Cys Pro

225 230 235 240
Ile Lys Pro Val Ala Val Arg Val Gly Gly Arg Arg Gln Ala Val Glu

245 250 255
Cys Ile Glu Asp Leu Leu Asn Glu Pro Gly Gln Pro Leu Asp Leu Ser

260 265 270
Cys Lys Arg Pro Arg Pro

275

<210> 38

<211> 128

<212> PRT

<213> Human adenovirus type 5

<400> 38

Met Ala Ala Ala Val Glu Ala Leu Tyr Val Val Leu Glu Arg Glu Gly

1 5 10 15

Ala Ile Leu Pro Arg Gln Glu Gly Phe Ser Gly Val Tyr Val Phe Phe

20 25 30
 Ser Pro Ile Asn Phe Val Ile Pro Pro Met Gly Ala Val Met Leu Ser
 35 40 45
 Leu Arg Leu Arg Val Cys Ile Pro Pro Gly Tyr Phe Gly Arg Phe Leu
 50 55 60
 Ala Leu Thr Asp Val Asn Gln Pro Asp Val Phe Thr Glu Ser Tyr Ile
 65 70 75 80
 Met Thr Pro Asp Met Thr Glu Glu Leu Ser Val Val Leu Phe Asn His

85 90 95
 Gly Asp Gln Phe Phe Tyr Gly His Ala Gly Met Ala Val Val Arg Leu
 100 105 110
 Met Leu Ile Arg Val Val Phe Pro Val Val Arg Gln Ala Ser Asn Val
 115 120 125

<210> 39

<211> 125

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic polypeptide

<400> 39

Met Ala Ala Ala Val Glu Ala Leu Tyr Val Val Leu Glu Arg Glu Gly

1 5 10 15
 Ala Ile Leu Pro Arg Gln Glu Gly Phe Ser Gly Val Tyr Val Phe Phe
 20 25 30
 Ser Pro Ile Asn Phe Val Ile Pro Pro Met Gly Ala Val Met Leu Ser
 35 40 45
 Leu Arg Leu Arg Val Cys Ile Pro Pro Gly Tyr Phe Gly Arg Phe Leu
 50 55 60
 Ala Leu Thr Asp Val Asn Gln Pro Asp Val Phe Thr Glu Ser Tyr Ile
 65 70 75 80
 Met Thr Pro Asp Met Thr Glu Glu Leu Ser Val Val Leu Phe Asn His
 85 90 95
 Gly Asp Gln Phe Phe Tyr Gly His Ala Gly Met Ala Val Val Arg Leu

100 105 110
 Met Leu Ile Arg Val Val Phe Pro Val Val Arg Gln Ala
 115 120 125
 <210> 40
 <211> 150
 <212> PRT
 <213> Human adenovirus type 5
 <
 400> 40
 Met Thr Thr Ser Gly Val Pro Phe Gly Met Thr Leu Arg Pro Thr Arg
 1 5 10 15
 Ser Arg Leu Ser Arg Arg Thr Pro Tyr Ser Arg Asp Arg Leu Pro Pro
 20 25 30
 Phe Glu Thr Glu Thr Arg Ala Thr Ile Leu Glu Asp His Pro Leu Leu
 35 40 45
 Pro Glu Cys Asn Thr Leu Thr Met His Asn Ala Trp Thr Ser Pro Ser
 50 55 60
 Pro Pro Val Lys Gln Pro Gln Val Gly Gln Gln Pro Val Ala Gln Gln
 65 70 75 80
 Leu Asp Ser Asp Met Asn Leu Ser Glu Leu Pro Gly Glu Phe Ile Asn
 85 90 95
 Ile Thr Asp Glu Arg Leu Ala Arg Gln Glu Thr Val Trp Asn Ile Thr
 100 105 110
 Pro Lys Asn Met Ser Val Thr His Asp Met Met Leu Phe Lys Ala Ser
 115 120 125
 Arg Gly Glu Arg Thr Val Tyr Ser Val Cys Trp Glu Gly Gly Gly Arg
 130 135 140
 Leu Asn Thr Arg Val Leu
 145 150
 <210> 41
 <211> 581
 <212> PRT
 <213> Human adenovirus type 5

<400> 41

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro

1 5 10 15

Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro

20 25 30

Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser

35 40 45

Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu

50 55 60

Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser

65 70 75 80

Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn

85 90 95

Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu

100 105 110

Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr

115 120 125

Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile

130 135 140

Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln

145 150 155 160

Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr

165 170 175

Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu

180 185 190

Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly

195 200 205

Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr

210 215 220

Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr

225 230 235 240

Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
245 250 255

Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
260 265 270

Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
275 280 285

Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn
290 295 300

Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
305 310 315 320

Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
325 330 335

Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
340 345 350

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
355 360 365

Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
370 375 380

Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400

Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415

Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430

Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
435 440 445

Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
450 455 460

Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
465 470 475 480

Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly

485 490 495
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
500 505 510
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
515 520 525
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
530 535 540

Thr Thr Pro Ser Ala Tyr Ser Met Ser Phe Ser Trp Asp Trp Ser Gly
545 550 555 560
His Asn Tyr Ile Asn Glu Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser
565 570 575
Tyr Ile Ala Gln Glu
580

<210> 42

<211> 671

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic polypeptide

<400> 42

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
1 5 10 15

Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
20 25 30
Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser
35 40 45
Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
50 55 60
Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
65 70 75 80

Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
85 90 95
Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu

100	105	110
Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr		
115	120	125
Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile		
130	135	140
Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln		
145	150	155
Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr		
165	170	175
Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu		
180	185	190
Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly		
195	200	205
Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr		
210	215	220
Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr		
225	230	235
Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala		
245	250	255
Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val		
260	265	270
Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln		
275	280	285
Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn		
290	295	300
Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu		
305	310	315
Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile		
325	330	335
Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro		
340	345	350

Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp
 355 360 365
 Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
 370 375 380
 Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
 385 390 395 400

 Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
 405 410 415
 Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
 420 425 430
 Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
 435 440 445
 Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
 450 455 460

 Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
 465 470 475 480
 Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
 485 490 495
 Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
 500 505 510
 Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
 515 520 525

 Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
 530 535 540
 Thr Thr Glu Met Trp His Glu Gly Leu Glu Glu Ala Ser Arg Leu Tyr
 545 550 555 560
 Phe Gly Glu Arg Asn Val Lys Gly Met Phe Glu Val Leu Glu Pro Leu
 565 570 575
 His Ala Met Met Glu Arg Gly Pro Gln Thr Leu Lys Glu Thr Ser Phe
 580 585 590

 Asn Gln Ala Tyr Gly Arg Asp Leu Met Glu Ala Gln Glu Trp Cys Arg

595 600 605
 Lys Tyr Met Lys Ser Gly Asn Val Lys Asp Leu Thr Gln Ala Trp Asp
 610 615 620
 Leu Tyr Tyr His Val Phe Arg Arg Ile Ser Lys Gln Pro Ser Ala Tyr
 625 630 635 640
 Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn Tyr Ile Asn Glu
 645 650 655

Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile Ala Gln Glu
 660 665 670

<210> 43

<211> 671

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic polypeptide

<400> 43

Met Lys Arg Ala Arg Pro Ser Glu Asp Thr Phe Asn Pro Val Tyr Pro
 1 5 10 15
 Tyr Asp Thr Glu Thr Gly Pro Pro Thr Val Pro Phe Leu Thr Pro Pro
 20 25 30
 Phe Val Ser Pro Asn Gly Phe Gln Glu Ser Pro Pro Gly Val Leu Ser

35 40 45
 Leu Arg Leu Ser Glu Pro Leu Val Thr Ser Asn Gly Met Leu Ala Leu
 50 55 60
 Lys Met Gly Asn Gly Leu Ser Leu Asp Glu Ala Gly Asn Leu Thr Ser
 65 70 75 80
 Gln Asn Val Thr Thr Val Ser Pro Pro Leu Lys Lys Thr Lys Ser Asn
 85 90 95
 Ile Asn Leu Glu Ile Ser Ala Pro Leu Thr Val Thr Ser Glu Ala Leu

100 105 110
 Thr Val Ala Ala Ala Ala Pro Leu Met Val Ala Gly Asn Thr Leu Thr
 115 120 125
 Met Gln Ser Gln Ala Pro Leu Thr Val His Asp Ser Lys Leu Ser Ile

130 135 140
 Ala Thr Gln Gly Pro Leu Thr Val Ser Glu Gly Lys Leu Ala Leu Gln
 145 150 155 160
 Thr Ser Gly Pro Leu Thr Thr Thr Asp Ser Ser Thr Leu Thr Ile Thr

 165 170 175
 Ala Ser Pro Pro Leu Thr Thr Ala Thr Gly Ser Leu Gly Ile Asp Leu
 180 185 190
 Lys Glu Pro Ile Tyr Thr Gln Asn Gly Lys Leu Gly Leu Lys Tyr Gly
 195 200 205
 Ala Pro Leu His Val Thr Asp Asp Leu Asn Thr Leu Thr Val Ala Thr
 210 215 220
 Gly Pro Gly Val Thr Ile Asn Asn Thr Ser Leu Gln Thr Lys Val Thr

 225 230 235 240
 Gly Ala Leu Gly Phe Asp Ser Gln Gly Asn Met Gln Leu Asn Val Ala
 245 250 255
 Gly Gly Leu Arg Ile Asp Ser Gln Asn Arg Arg Leu Ile Leu Asp Val
 260 265 270
 Ser Tyr Pro Phe Asp Ala Gln Asn Gln Leu Asn Leu Arg Leu Gly Gln
 275 280 285
 Gly Pro Leu Phe Ile Asn Ser Ala His Asn Leu Asp Ile Asn Tyr Asn

 290 295 300
 Lys Gly Leu Tyr Leu Phe Thr Ala Ser Asn Asn Ser Lys Lys Leu Glu
 305 310 315 320
 Val Asn Leu Ser Thr Ala Lys Gly Leu Met Phe Asp Ala Thr Ala Ile
 325 330 335
 Ala Ile Asn Ala Gly Asp Gly Leu Glu Phe Gly Ser Pro Asn Ala Pro
 340 345 350
 Asn Thr Asn Pro Leu Lys Thr Lys Ile Gly His Gly Leu Glu Phe Asp

 355 360 365
 Ser Asn Lys Ala Met Val Pro Lys Leu Gly Thr Gly Leu Ser Phe Asp
 370 375 380

Ser Thr Gly Ala Ile Thr Val Gly Asn Lys Asn Asn Asp Lys Leu Thr
385 390 395 400
Leu Trp Thr Thr Pro Ala Pro Ser Pro Asn Cys Arg Leu Asn Ala Glu
405 410 415
Lys Asp Ala Lys Leu Thr Leu Val Leu Thr Lys Cys Gly Ser Gln Ile
420 425 430
Leu Ala Thr Val Ser Val Leu Ala Val Lys Gly Ser Leu Ala Pro Ile
435 440 445
Ser Gly Thr Val Gln Ser Ala His Leu Ile Ile Arg Phe Asp Glu Asn
450 455 460
Gly Val Leu Leu Asn Asn Ser Phe Leu Asp Pro Glu Tyr Trp Asn Phe
465 470 475 480
Arg Asn Gly Asp Leu Thr Glu Gly Thr Ala Tyr Thr Asn Ala Val Gly
485 490 495
Phe Met Pro Asn Leu Ser Ala Tyr Pro Lys Ser His Gly Lys Thr Ala
500 505 510
Lys Ser Asn Ile Val Ser Gln Val Tyr Leu Asn Gly Asp Lys Thr Lys
515 520 525
Pro Val Thr Leu Thr Ile Thr Leu Asn Gly Thr Gln Glu Thr Gly Asp
530 535 540
Thr Thr Glu Met Trp His Glu Gly Leu Glu Glu Ala Ser Arg Leu Tyr
545 550 555 560
Phe Gly Glu Arg Asn Val Lys Gly Met Phe Glu Val Leu Glu Pro Leu
565 570 575
His Ala Met Met Glu Arg Gly Pro Gln Thr Leu Lys Glu Thr Ser Phe
580 585 590
Asn Gln Ala Tyr Gly Arg Asp Leu Met Glu Ala Gln Glu Trp Cys Arg
595 600 605
Lys Tyr Met Lys Ser Gly Asn Val Lys Asp Leu Leu Gln Ala Trp Asp
610 615 620
Leu Tyr Tyr His Val Phe Arg Arg Ile Ser Lys Gln Pro Ser Ala Tyr

625 630 635 640
Ser Met Ser Phe Ser Trp Asp Trp Ser Gly His Asn Tyr Ile Asn Glu

645 650 655
Ile Phe Ala Thr Ser Ser Tyr Thr Phe Ser Tyr Ile Ala Gln Glu

660 665 670

<210> 44

<211> 238

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic polypeptide

<400> 44

Met Ala Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly

1 5 10 15

Gly Ser Leu Arg Leu Thr Cys Ala Ala Ser Gly Arg Thr Ser Arg Ser

20 25 30

Tyr Gly Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe

35 40 45

Val Ser Gly Ile Ser Trp Arg Gly Asp Ser Thr Gly Tyr Ala Asp Ser

50 55 60

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val

65 70 75 80

Asp Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Ile Tyr Tyr

85 90 95

Cys Ala Ala Ala Ala Gly Ser Thr Trp Tyr Gly Thr Leu Tyr Glu Tyr

100 105 110

Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Ser Gly

115 120 125

Ser Gly Ser Thr Gly Val Gln Val Glu Thr Ile Ser Pro Gly Asp Gly

130 135 140

Arg Thr Phe Pro Lys Arg Gly Gln Thr Cys Val Val His Tyr Thr Gly

145 150 155 160

Met Leu Glu Asp Gly Lys Lys Phe Asp Ser Ser Arg Asp Arg Asn Lys

165 170 175
Pro Phe Lys Phe Met Leu Gly Lys Gln Glu Val Ile Arg Gly Trp Glu

180 185 190
Glu Gly Val Ala Gln Met Ser Val Gly Gln Arg Ala Lys Leu Thr Ile

195 200 205
Ser Pro Asp Tyr Ala Tyr Gly Ala Thr Gly His Pro Gly Ile Ile Pro

210 215 220
Pro His Ala Thr Leu Val Phe Asp Val Glu Leu Leu Lys Leu

225 230 235

<210> 45

<211> 952

<212> PRT

<213> Human adenovirus type 5

<400> 45

Met Ala Thr Pro Ser Met Met Pro Gln Trp Ser Tyr Met His Ile Ser

1 5 10 15

Gly Gln Asp Ala Ser Glu Tyr Leu Ser Pro Gly Leu Val Gln Phe Ala

20 25 30

Arg Ala Thr Glu Thr Tyr Phe Ser Leu Asn Asn Lys Phe Arg Asn Pro

35 40 45

Thr Val Ala Pro Thr His Asp Val Thr Thr Asp Arg Ser Gln Arg Leu

50 55 60

Thr Leu Arg Phe Ile Pro Val Asp Arg Glu Asp Thr Ala Tyr Ser Tyr

65 70 75 80

Lys Ala Arg Phe Thr Leu Ala Val Gly Asp Asn Arg Val Leu Asp Met

85 90 95

Ala Ser Thr Tyr Phe Asp Ile Arg Gly Val Leu Asp Arg Gly Pro Thr

100 105 110

Phe Lys Pro Tyr Ser Gly Thr Ala Tyr Asn Ala Leu Ala Pro Lys Gly

115 120 125

Ala Pro Asn Pro Cys Glu Trp Asp Glu Ala Ala Thr Ala Leu Glu Ile

130	135	140	
Asn Leu Glu Glu Glu Asp Asp Asp Asn Glu Asp Glu Val Asp Glu Gln			
145	150	155	160
Ala Glu Gln Gln Lys Thr His Val Phe Gly Gln Ala Pro Tyr Ser Gly			
	165	170	175
Ile Asn Ile Thr Lys Glu Gly Ile Gln Ile Gly Val Glu Gly Gln Thr			
	180	185	190
Pro Lys Tyr Ala Asp Lys Thr Phe Gln Pro Glu Pro Gln Ile Gly Glu			
	195	200	205
Ser Gln Trp Tyr Glu Thr Glu Ile Asn His Ala Ala Gly Arg Val Leu			
	210	215	220
Lys Lys Thr Thr Pro Met Lys Pro Cys Tyr Gly Ser Tyr Ala Lys Pro			
225	230	235	240
Thr Asn Glu Asn Gly Gly Gln Gly Ile Leu Val Lys Gln Gln Asn Gly			
	245	250	255
Lys Leu Glu Ser Gln Val Glu Met Gln Phe Phe Ser Thr Thr Glu Ala			
	260	265	270
Thr Ala Gly Asn Gly Asp Asn Leu Thr Pro Lys Val Val Leu Tyr Ser			
	275	280	285
Glu Asp Val Asp Ile Glu Thr Pro Asp Thr His Ile Ser Tyr Met Pro			
	290	295	300
Thr Ile Lys Glu Gly Asn Ser Arg Glu Leu Met Gly Gln Gln Ser Met			
305	310	315	320
Pro Asn Arg Pro Asn Tyr Ile Ala Phe Arg Asp Asn Phe Ile Gly Leu			
	325	330	335
Met Tyr Tyr Asn Ser Thr Gly Asn Met Gly Val Leu Ala Gly Gln Ala			
	340	345	350
Ser Gln Leu Asn Ala Val Val Asp Leu Gln Asp Arg Asn Thr Glu Leu			
	355	360	365
Ser Tyr Gln Leu Leu Leu Asp Ser Ile Gly Asp Arg Thr Arg Tyr Phe			
	370	375	380

Ser Met Trp Asn Gln Ala Val Asp Ser Tyr Asp Pro Asp Val Arg Ile
385 390 395 400
Ile Glu Asn His Gly Thr Glu Asp Glu Leu Pro Asn Tyr Cys Phe Pro
405 410 415
Leu Gly Gly Val Ile Asn Thr Glu Thr Leu Thr Lys Val Lys Pro Lys
420 425 430
Thr Gly Gln Glu Asn Gly Trp Glu Lys Asp Ala Thr Glu Phe Ser Asp
435 440 445

Lys Asn Glu Ile Arg Val Gly Asn Asn Phe Ala Met Glu Ile Asn Leu
450 455 460
Asn Ala Asn Leu Trp Arg Asn Phe Leu Tyr Ser Asn Ile Ala Leu Tyr
465 470 475 480
Leu Pro Asp Lys Leu Lys Tyr Ser Pro Ser Asn Val Lys Ile Ser Asp
485 490 495
Asn Pro Asn Thr Tyr Asp Tyr Met Asn Lys Arg Val Val Ala Pro Gly
500 505 510

Leu Val Asp Cys Tyr Ile Asn Leu Gly Ala Arg Trp Ser Leu Asp Tyr
515 520 525
Met Asp Asn Val Asn Pro Phe Asn His His Arg Asn Ala Gly Leu Arg
530 535 540
Tyr Arg Ser Met Leu Leu Gly Asn Gly Arg Tyr Val Pro Phe His Ile
545 550 555 560
Gln Val Pro Gln Lys Phe Phe Ala Ile Lys Asn Leu Leu Leu Leu Pro
565 570 575

Gly Ser Tyr Thr Tyr Glu Trp Asn Phe Arg Lys Asp Val Asn Met Val
580 585 590
Leu Gln Ser Ser Leu Gly Asn Asp Leu Arg Val Asp Gly Ala Ser Ile
595 600 605
Lys Phe Asp Ser Ile Cys Leu Tyr Ala Thr Phe Phe Pro Met Ala His
610 615 620
Asn Thr Ala Ser Thr Leu Glu Ala Met Leu Arg Asn Asp Thr Asn Asp

625				630				635				640			
Gln	Ser	Phe	Asn	Asp	Tyr	Leu	Ser	Ala	Ala	Asn	Met	Leu	Tyr	Pro	Ile
				645				650				655			
Pro	Ala	Asn	Ala	Thr	Asn	Val	Pro	Ile	Ser	Ile	Pro	Ser	Arg	Asn	Trp
				660				665				670			
Ala	Ala	Phe	Arg	Gly	Trp	Ala	Phe	Thr	Arg	Leu	Lys	Thr	Lys	Glu	Thr
				675				680				685			
Pro	Ser	Leu	Gly	Ser	Gly	Tyr	Asp	Pro	Tyr	Tyr	Thr	Tyr	Ser	Gly	Ser
690				695				700							
Ile	Pro	Tyr	Leu	Asp	Gly	Thr	Phe	Tyr	Leu	Asn	His	Thr	Phe	Lys	Lys
705				710				715				720			
Val	Ala	Ile	Thr	Phe	Asp	Ser	Ser	Val	Ser	Trp	Pro	Gly	Asn	Asp	Arg
				725				730				735			
Leu	Leu	Thr	Pro	Asn	Glu	Phe	Glu	Ile	Lys	Arg	Ser	Val	Asp	Gly	Glu
				740				745				750			
Gly	Tyr	Asn	Val	Ala	Gln	Cys	Asn	Met	Thr	Lys	Asp	Trp	Phe	Leu	Val
755				760				765							
Gln	Met	Leu	Ala	Asn	Tyr	Asn	Ile	Gly	Tyr	Gln	Gly	Phe	Tyr	Ile	Pro
770				775				780							
Glu	Ser	Tyr	Lys	Asp	Arg	Met	Tyr	Ser	Phe	Phe	Arg	Asn	Phe	Gln	Pro
785				790				795				800			
Met	Ser	Arg	Gln	Val	Val	Asp	Asp	Thr	Lys	Tyr	Lys	Asp	Tyr	Gln	Gln
				805				810				815			
Val	Gly	Ile	Leu	His	Gln	His	Asn	Asn	Ser	Gly	Phe	Val	Gly	Tyr	Leu
				820				825				830			
Ala	Pro	Thr	Met	Arg	Glu	Gly	Gln	Ala	Tyr	Pro	Ala	Asn	Phe	Pro	Tyr
835				840				845							
Pro	Leu	Ile	Gly	Lys	Thr	Ala	Val	Asp	Ser	Ile	Thr	Gln	Lys	Lys	Phe
850				855				860							
Leu	Cys	Asp	Arg	Thr	Leu	Trp	Arg	Ile	Pro	Phe	Ser	Ser	Asn	Phe	Met
865				870				875				880			

Ser Met Gly Ala Leu Thr Asp Leu Gly Gln Asn Leu Leu Tyr Ala Asn
885 890 895

Ser Ala His Ala Leu Asp Met Thr Phe Glu Val Asp Pro Met Asp Glu
900 905 910

Pro Thr Leu Leu Tyr Val Leu Phe Glu Val Phe Asp Val Val Arg Val
915 920 925

His Arg Pro His Arg Gly Val Ile Glu Thr Val Tyr Leu Arg Thr Pro
930 935 940

Phe Ser Ala Gly Asn Ala Thr Thr
945 950

<210> 46

<211> 952

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic polypeptide

<400> 46

Met Ala Thr Pro Ser Met Met Pro Gln Trp Ser Tyr Met His Ile Ser
1 5 10 15

Gly Gln Asp Ala Ser Glu Tyr Leu Ser Pro Gly Leu Val Gln Phe Ala
20 25 30

Arg Ala Thr Glu Thr Tyr Phe Ser Leu Asn Asn Lys Phe Arg Asn Pro
35 40 45

Thr Val Ala Pro Thr His Asp Val Thr Thr Asp Arg Ser Gln Arg Leu

50 55 60

Thr Leu Arg Phe Ile Pro Val Asp Arg Glu Asp Thr Ala Tyr Ser Tyr
65 70 75 80

Lys Ala Arg Phe Thr Leu Ala Val Gly Asp Asn Arg Val Leu Asp Met
85 90 95

Ala Ser Thr Tyr Phe Asp Ile Arg Gly Val Leu Asp Arg Gly Pro Thr
100 105 110

Phe Lys Pro Tyr Ser Gly Thr Ala Tyr Asn Ala Leu Ala Pro Lys Gly

115	120	125	
Ala Pro Asn Pro Cys Glu Trp Asp Glu Ala Ala Thr Ala Leu Glu Ile			
130	135	140	
Asn Leu Glu Glu Glu Asp Asp Asp Asn Glu Asp Glu Val Asp Glu Gln			
145	150	155	160
Ala Glu Gln Gln Lys Thr His Val Phe Gly Gln Ala Pro Tyr Ser Gly			
165	170	175	
Ile Asn Ile Thr Lys Glu Gly Ile Gln Ile Gly Val Glu Gly Gln Thr			
180	185	190	
Pro Lys Tyr Ala Asp Lys Thr Phe Gln Pro Glu Pro Gln Ile Gly Glu			
195	200	205	
Ser Gln Trp Tyr Glu Thr Glu Ile Asn His Ala Ala Gly Arg Val Leu			
210	215	220	
Lys Lys Thr Thr Pro Met Lys Pro Cys Tyr Gly Ser Tyr Ala Lys Pro			
225	230	235	240
Thr Asn Glu Asn Gly Gly Gln Gly Ile Leu Val Lys Gln Gln Asn Gly			
245	250	255	
Lys Leu Glu Ser Gln Val Glu Met Gln Phe Phe Ser Thr Thr Glu Ala			
260	265	270	
Thr Ala Gly Asn Gly Asp Asn Leu Thr Pro Lys Val Val Leu Tyr Ser			
275	280	285	
Glu Asp Val Asp Ile Glu Thr Pro Asp Thr His Ile Ser Tyr Met Pro			
290	295	300	
Thr Ile Lys Glu Gly Asn Ser Arg Glu Leu Met Gly Gln Gln Ser Met			
305	310	315	320
Pro Asn Arg Pro Asn Tyr Ile Ala Phe Arg Asp Asn Phe Ile Gly Leu			
325	330	335	
Met Tyr Tyr Asn Ser Thr Gly Asn Met Gly Val Leu Ala Gly Gln Ala			
340	345	350	
Ser Gln Leu Asn Ala Val Val Asp Leu Gln Asp Arg Asn Thr Glu Leu			
355	360	365	

Ser Tyr Gln Leu Leu Leu Asp Ser Ile Gly Asp Arg Thr Arg Tyr Phe

370 375 380

Ser Met Trp Asn Gln Ala Val Asp Ser Tyr Asp Pro Asp Val Arg Ile

385 390 395 400

Ile Glu Asn His Gly Thr Glu Asp Glu Leu Pro Asn Tyr Cys Phe Pro

405 410 415

Leu Gly Gly Val Ile Asn Thr Glu Thr Leu Thr Lys Val Lys Pro Lys

420 425 430

Thr Gly Gln Glu Asn Gly Trp Glu Lys Asp Ala Thr Glu Phe Ser Asp

435 440 445

Lys Asn Gln Ile Arg Val Gly Asn Asn Phe Ala Met Glu Ile Asn Leu

450 455 460

Asn Ala Asn Leu Trp Arg Asn Phe Leu Tyr Ser Asn Ile Ala Leu Tyr

465 470 475 480

Leu Pro Asp Lys Leu Lys Tyr Ser Pro Ser Asn Val Lys Ile Ser Asp

485 490 495

Asn Pro Asn Thr Tyr Asp Tyr Met Asn Lys Arg Val Val Ala Pro Gly

500 505 510

Leu Val Asp Cys Tyr Ile Asn Leu Gly Ala Arg Trp Ser Leu Asp Tyr

515 520 525

Met Asp Asn Val Asn Pro Phe Asn His His Arg Asn Ala Gly Leu Arg

530 535 540

Tyr Arg Ser Met Leu Leu Gly Asn Gly Arg Tyr Val Pro Phe His Ile

545 550 555 560

Gln Val Pro Gln Lys Phe Phe Ala Ile Lys Asn Leu Leu Leu Leu Pro

565 570 575

Gly Ser Tyr Thr Tyr Glu Trp Asn Phe Arg Lys Asp Val Asn Met Val

580 585 590

Leu Gln Ser Ser Leu Gly Asn Asp Leu Arg Val Asp Gly Ala Ser Ile

595 600 605

Lys Phe Asp Ser Ile Cys Leu Tyr Ala Thr Phe Phe Pro Met Ala His

610 615 620
 Asn Thr Ala Ser Thr Leu Glu Ala Met Leu Arg Asn Asp Thr Asn Asp

 625 630 635 640
 Gln Ser Phe Asn Asp Tyr Leu Ser Ala Ala Asn Met Leu Tyr Pro Ile

 645 650 655
 Pro Ala Asn Ala Thr Asn Val Pro Ile Ser Ile Pro Ser Arg Asn Trp

 660 665 670
 Ala Ala Phe Arg Gly Trp Ala Phe Thr Arg Leu Lys Thr Lys Glu Thr

 675 680 685
 Pro Ser Leu Gly Ser Gly Tyr Asp Pro Tyr Tyr Thr Tyr Ser Gly Ser

 690 695 700
 Ile Pro Tyr Leu Asp Gly Thr Phe Tyr Leu Asn His Thr Phe Lys Lys
 705 710 715 720
 Val Ala Ile Thr Phe Asp Ser Ser Val Ser Trp Pro Gly Asn Asp Arg

 725 730 735
 Leu Leu Thr Pro Asn Glu Phe Glu Ile Lys Arg Ser Val Asp Gly Glu

 740 745 750
 Gly Tyr Asn Val Ala Gln Cys Asn Met Thr Lys Asp Trp Phe Leu Val

 755 760 765
 Gln Met Leu Ala Asn Tyr Asn Ile Gly Tyr Gln Gly Phe Tyr Ile Pro
 770 775 780
 Glu Ser Tyr Lys Asp Arg Met Tyr Ser Phe Phe Arg Asn Phe Gln Pro
 785 790 795 800
 Met Ser Arg Gln Val Val Asp Asp Thr Lys Tyr Lys Asp Tyr Gln Gln

 805 810 815
 Val Gly Ile Leu His Gln His Asn Asn Ser Gly Phe Val Gly Tyr Leu

 820 825 830
 Ala Pro Thr Met Arg Glu Gly Gln Ala Tyr Pro Ala Asn Phe Pro Tyr

 835 840 845
 Pro Leu Ile Gly Lys Thr Ala Val Asp Ser Ile Thr Gln Lys Lys Phe

 850 855 860

Leu Cys Asp Arg Thr Leu Trp Arg Ile Pro Phe Ser Ser Asn Phe Met
 865 870 875 880
 Ser Met Gly Ala Leu Thr Asp Leu Gly Gln Asn Leu Leu Tyr Ala Asn

885 890 895
 Ser Ala His Ala Leu Asp Met Thr Phe Glu Val Asp Pro Met Asp Glu
 900 905 910
 Pro Thr Leu Leu Tyr Val Leu Phe Glu Val Phe Asp Val Val Arg Val
 915 920 925
 His Arg Pro His Arg Gly Val Ile Glu Thr Val Tyr Leu Arg Thr Pro
 930 935 940
 Phe Ser Ala Gly Asn Ala Thr Thr
 945 950

<210> 47

<211> 266

<212> PRT

<213> Human adenovirus type 12

<400> 47

Met Arg Thr Glu Met Thr Pro Leu Val Leu Ser Tyr Gln Glu Ala Asp
 1 5 10 15
 Asp Ile Leu Glu His Leu Val Asp Asn Phe Phe Asn Glu Val Pro Ser
 20 25 30
 Asp Asp Asp Leu Tyr Val Pro Ser Leu Tyr Glu Leu Tyr Asp Leu Asp
 35 40 45
 Val Glu Ser Ala Gly Glu Asp Asn Asn Glu Gln Ala Val Asn Glu Phe

50 55 60
 Phe Pro Glu Ser Leu Ile Leu Ala Ala Ser Glu Gly Leu Phe Leu Pro
 65 70 75 80
 Glu Pro Pro Val Leu Ser Pro Val Cys Glu Pro Ile Gly Gly Glu Cys
 85 90 95
 Met Pro Gln Leu His Pro Glu Asp Met Asp Leu Leu Cys Tyr Glu Met
 100 105 110

Gly Phe Pro Cys Ser Asp Ser Glu Asp Glu Gln Asp Glu Asn Gly Met

115 120 125
Ala His Val Ser Ala Ser Ala Ala Ala Ala Ala Asp Arg Glu Arg
130 135 140
Glu Glu Phe Gln Leu Asp His Pro Glu Leu Pro Gly His Asn Cys Lys
145 150 155 160
Ser Cys Glu His His Arg Asn Ser Thr Gly Asn Thr Asp Leu Met Cys
165 170 175
Ser Leu Cys Tyr Leu Arg Ala Tyr Asn Met Phe Ile Tyr Ser Pro Val

180 185 190
Ser Asp Asn Glu Pro Glu Pro Asn Ser Thr Leu Asp Gly Asp Glu Arg
195 200 205
Pro Ser Pro Pro Lys Leu Gly Ser Ala Val Pro Glu Gly Val Ile Lys
210 215 220
Pro Val Pro Gln Arg Val Thr Gly Arg Arg Arg Cys Ala Val Glu Ser
225 230 235 240
Ile Leu Asp Leu Ile Gln Glu Glu Glu Arg Glu Gln Thr Val Pro Val

245 250 255
Asp Leu Ser Val Lys Arg Pro Arg Cys Asn

260 265

<210> 48

<211> 261

<212> PRT

<213> Human adenovirus type 7

<400> 48

Met Arg His Leu Arg Phe Leu Pro Gln Glu Ile Ile Ser Ser Glu Thr

1 5 10 15

Gly Ile Glu Ile Leu Glu Phe Val Val Asn Thr Leu Met Gly Asp Asp

20 25 30

Pro Glu Pro Pro Val Gln Pro Phe Asp Pro Pro Thr Leu His Asp Leu

35

40

45

Tyr Asp Leu Glu Val Asp Gly Pro Glu Asp Pro Asn Glu Gly Ala Val
 50 55 60
 Asn Gly Phe Phe Thr Asp Ser Met Leu Leu Ala Ala Asp Glu Gly Leu
 65 70 75 80
 Asp Ile Asn Pro Pro Pro Glu Thr Leu Val Thr Pro Gly Val Val Val
 85 90 95
 Glu Ser Gly Arg Gly Gly Lys Lys Leu Pro Asp Leu Gly Ala Ala Glu
 100 105 110
 Met Asp Leu Arg Cys Tyr Glu Glu Gly Phe Pro Pro Ser Asp Asp Glu
 115 120 125
 Asp Gly Glu Thr Glu Gln Ser Ile His Thr Ala Val Asn Glu Gly Val
 130 135 140
 Lys Ala Ala Ser Asp Val Phe Lys Leu Asp Cys Pro Glu Leu Pro Gly
 145 150 155 160
 His Gly Cys Lys Ser Cys Glu Phe His Arg Asn Asn Thr Gly Met Lys
 165 170 175
 Glu Leu Leu Cys Ser Leu Cys Tyr Met Arg Met His Cys His Phe Ile
 180 185 190
 Tyr Ser Pro Val Ser Asp Asp Glu Ser Pro Ser Pro Asp Ser Thr Thr
 195 200 205
 Ser Pro Pro Glu Ile Gln Ala Pro Ala Pro Ala Asn Val Cys Lys Pro
 210 215 220
 Ile Pro Val Lys Pro Lys Pro Gly Lys Arg Pro Ala Val Asp Lys Leu
 225 230 235 240
 Glu Asp Leu Leu Glu Gly Gly Asp Gly Pro Leu Asp Leu Ser Thr Arg
 245 250 255
 Lys Leu Pro Arg Gln
 260

<210> 49

<211> 289

<212> PRT

<213> Human adenovirus type 2

<400> 49

Met Arg His Ile Ile Cys His Gly Gly Val Ile Thr Glu Glu Met Ala

1 5 10 15

Ala Ser Leu Leu Asp Gln Leu Ile Glu Glu Val Leu Ala Asp Asn Leu

20 25 30

Pro Pro Pro Ser His Phe Glu Pro Pro Thr Leu His Glu Leu Tyr Asp

35 40 45

Leu Asp Val Thr Ala Pro Glu Asp Pro Asn Glu Glu Ala Val Ser Gln

50 55 60

Ile Phe Pro Asp Ser Val Met Leu Ala Val Gln Glu Gly Ile Asp Leu

65 70 75 80

Leu Thr Phe Pro Pro Ala Pro Gly Ser Pro Glu Pro Pro His Leu Ser

85 90 95

Arg Gln Pro Glu Gln Pro Glu Gln Arg Ala Leu Gly Pro Val Ser Met

100 105 110

Pro Asn Leu Val Pro Glu Val Ile Asp Leu Thr Cys His Glu Ala Gly

115 120 125

Phe Pro Pro Ser Asp Asp Glu Asp Glu Glu Gly Glu Glu Phe Val Leu

130 135 140

Asp Tyr Val Glu His Pro Gly His Gly Cys Arg Ser Cys His Tyr His

145 150 155 160

Arg Arg Asn Thr Gly Asp Pro Asp Ile Met Cys Ser Leu Cys Tyr Met

165 170 175

Arg Thr Cys Gly Met Phe Val Tyr Ser Pro Val Ser Glu Pro Glu Pro

180 185 190

Glu Pro Glu Pro Glu Pro Glu Pro Ala Arg Pro Thr Arg Arg Pro Lys

195 200 205

Leu Val Pro Ala Ile Leu Arg Arg Pro Thr Ser Pro Val Ser Arg Glu

210 215 220

Cys Asn Ser Ser Thr Asp Ser Cys Asp Ser Gly Pro Ser Asn Thr Pro

225 230 235 240

Pro Glu Ile His Pro Val Val Pro Leu Cys Pro Ile Lys Pro Val Ala
245 250 255

Val Arg Val Gly Gly Arg Arg Gln Ala Val Glu Cys Ile Glu Asp Leu
260 265 270

Leu Asn Glu Ser Gly Gln Pro Leu Asp Leu Ser Cys Lys Arg Pro Arg
275 280 285

Pro

<210> 50

<211> 289

<212> PRT

<213> Human adenovirus type 5

<400> 50

Met Arg His Ile Ile Cys His Gly Gly Val Ile Thr Glu Glu Met Ala
1 5 10 15

Ala Ser Leu Leu Asp Gln Leu Ile Glu Glu Val Leu Ala Asp Asn Leu
20 25 30

Pro Pro Pro Ser His Phe Glu Pro Pro Thr Leu His Glu Leu Tyr Asp
35 40 45

Leu Asp Val Thr Ala Pro Glu Asp Pro Asn Glu Glu Ala Val Ser Gln
50 55 60

Ile Phe Pro Asp Ser Val Met Leu Ala Val Gln Glu Gly Ile Asp Leu
65 70 75 80

Leu Thr Phe Pro Pro Ala Pro Gly Ser Pro Glu Pro Pro His Leu Ser
85 90 95

Arg Gln Pro Glu Gln Pro Glu Gln Arg Ala Leu Gly Pro Val Ser Met
100 105 110

Pro Asn Leu Val Pro Glu Val Ile Asp Leu Thr Cys His Glu Ala Gly
115 120 125

Phe Pro Pro Ser Asp Asp Glu Asp Glu Glu Gly Glu Glu Phe Val Leu
130 135 140

Asp Tyr Val Glu His Pro Gly His Gly Cys Arg Ser Cys His Tyr His

145 150 155 160
 Arg Arg Asn Thr Gly Asp Pro Asp Ile Met Cys Ser Leu Cys Tyr Met
 165 170 175

Arg Thr Cys Gly Met Phe Val Tyr Ser Pro Val Ser Glu Pro Glu Pro
 180 185 190
 Glu Pro Glu Pro Glu Pro Glu Pro Ala Arg Pro Thr Arg Arg Pro Lys
 195 200 205
 Met Ala Pro Ala Ile Leu Arg Arg Pro Thr Ser Pro Val Ser Arg Glu
 210 215 220
 Cys Asn Ser Ser Thr Asp Ser Cys Asp Ser Gly Pro Ser Asn Thr Pro
 225 230 235 240

Pro Glu Ile His Pro Val Val Pro Leu Cys Pro Ile Lys Pro Val Ala
 245 250 255
 Val Arg Val Gly Gly Arg Arg Gln Ala Val Glu Cys Ile Glu Asp Leu
 260 265 270
 Leu Asn Glu Pro Gly Gln Pro Leu Asp Leu Ser Cys Lys Arg Pro Arg
 275 280 285
 Pro

<210> 51

<211> 251

<212> PRT

<213> Human adenovirus type 9

<400> 51

Met Arg His Leu Arg Leu Leu Pro Ser Thr Val Pro Gly Glu Leu Ala

1 5 10 15
 Val Leu Met Leu Glu Asp Phe Val Asp Thr Val Leu Glu Asp Glu Leu
 20 25 30
 His Pro Ser Pro Phe Glu Leu Gly Pro Thr Leu Gln Asp Leu Tyr Asp
 35 40 45
 Leu Glu Val Asp Ala His Asp Asp Asp Pro Asn Glu Glu Ala Val Asn
 50 55 60

Leu Ile Phe Pro Glu Ser Met Ile Leu Gln Ala Asp Ile Ala Asn Glu

65 70 75 80
Ser Thr Pro Leu His Thr Pro Thr Leu Ser Pro Ile Pro Glu Leu Glu
85 90 95
Glu Glu Asp Glu Leu Asp Leu Arg Cys Tyr Glu Glu Gly Phe Pro Pro
100 105 110
Ser Asp Ser Glu Asp Glu Arg Gly Glu Gln Thr Met Ala Leu Ile Ser
115 120 125
Asp Tyr Ala Cys Val Ile Val Glu Glu Gln Asp Val Ile Glu Lys Ser

130 135 140
Thr Glu Pro Val Gln Gly Cys Arg Asn Cys Gln Tyr His Arg Asp Lys
145 150 155 160
Ser Gly Asp Val Asn Ala Ser Cys Ala Leu Cys Tyr Met Lys Gln Thr
165 170 175
Phe Ser Phe Ile Tyr Ser Pro Val Ser Glu Asp Glu Leu Ser Pro Ser
180 185 190
Glu Glu Asp His Pro Ser Pro Pro Glu Leu Ser Gly Glu Thr Pro Leu

195 200 205
Gln Val Phe Arg Pro Thr Pro Val Arg Pro Ser Gly Glu Arg Arg Ala
210 215 220
Ala Val Asp Lys Ile Glu Asp Leu Leu Gln Asp Met Gly Gly Asp Glu
225 230 235 240
Pro Leu Asp Leu Ser Leu Lys Arg Pro Arg Asn
245 250

<210> 52

<211> 257

<212> PRT

<213> Human adenovirus type 4

<400> 52

Met Arg His Leu Arg Asp Leu Pro Asp Glu Glu Ile Ile Ile Ala Ser

1 5 10 15

Gly Ser Glu Ile Leu Glu Leu Val Val Asn Ala Met Met Gly Asp Asp
 20 25 30
 His Pro Glu Pro Pro Thr Pro Phe Glu Thr Pro Ser Leu His Asp Leu
 35 40 45
 Tyr Asp Leu Glu Val Asp Val Pro Glu Asp Asp Pro Asn Glu Lys Ala
 50 55 60
 Val Asn Asp Leu Phe Ser Asp Ala Ala Leu Leu Ala Ala Glu Glu Ala

 65 70 75 80
 Ser Ser Pro Ser Ser Asp Ser Asp Ser Ser Leu His Thr Pro Arg His
 85 90 95
 Asp Arg Gly Glu Lys Glu Ile Pro Gly Leu Lys Trp Glu Lys Met Asp
 100 105 110
 Leu Arg Cys Tyr Glu Glu Cys Leu Pro Pro Ser Asp Asp Glu Asp Glu
 115 120 125
 Gln Ala Ile Gln Asn Ala Ala Ser His Gly Val Gln Ala Val Ser Glu

 130 135 140
 Ser Phe Ala Leu Asp Cys Pro Pro Leu Pro Gly His Gly Cys Lys Ser
 145 150 155 160
 Cys Glu Phe His Arg Ile Asn Thr Gly Asp Lys Ala Val Leu Cys Ala
 165 170 175
 Leu Cys Tyr Met Arg Ala Tyr Asn His Cys Val Tyr Ser Pro Val Ser
 180 185 190
 Asp Ala Asp Asp Glu Thr Pro Thr Thr Glu Ser Thr Leu Ser Pro Pro

 195 200 205
 Glu Ile Gly Thr Ser Pro Ser Asp Asn Ile Val Arg Pro Val Pro Val
 210 215 220
 Arg Ala Thr Gly Arg Arg Ala Ala Val Glu Cys Leu Asp Asp Leu Leu
 225 230 235 240
 Gln Gly Gly Asp Glu Pro Leu Asp Leu Cys Thr Arg Lys Arg Pro Arg
 245 250 255
 His

<210> 53

<211> 249

<212> PRT

<213> Human adenovirus type 40

<400> 53

Met Arg Met Leu Pro Asp Phe Phe Thr Gly Asn Trp Asp Asp Met Phe

1 5 10 15

Gln Gly Leu Leu Glu Thr Glu Tyr Val Phe Asp Phe Pro Glu Pro Ser

20 25 30

Glu Ala Ser Glu Glu Met Ser Leu His Asp Leu Phe Asp Val Glu Val

35 40 45

Asp Gly Phe Glu Glu Asp Ala Asn Gln Glu Ala Val Asp Gly Met Phe

50 55 60

Pro Glu Arg Leu Leu Ser Glu Ala Glu Ser Ala Ala Glu Ser Gly Ser

65 70 75 80

Gly Asp Ser Gly Val Gly Glu Glu Leu Leu Pro Val Asp Leu Asp Leu

85 90 95

Lys Cys Tyr Glu Asp Gly Leu Pro Pro Ser Asp Pro Glu Thr Asp Glu

100 105 110

Ala Thr Glu Ala Glu Glu Glu Ala Ala Met Pro Thr Tyr Val Asn Glu

115 120 125

Asn Glu Asn Glu Leu Val Leu Asp Cys Pro Glu Asn Pro Gly Arg Gly

130 135 140

Cys Arg Ala Cys Asp Phe His Arg Gly Thr Ser Gly Asn Pro Glu Ala

145 150 155 160

Met Cys Ala Leu Cys Tyr Met Arg Leu Thr Gly His Cys Ile Tyr Ser

165 170 175

Pro Ile Ser Asp Ala Glu Gly Glu Ser Glu Ser Gly Ser Pro Glu Asp

180 185 190

Thr Asp Phe Pro His Pro Leu Thr Ala Thr Pro Pro His Gly Ile Val

195 200 205

Arg Thr Ile Pro Cys Arg Val Ser Cys Arg Arg Arg Pro Ala Val Glu
 210 215 220
 Cys Ile Glu Asp Leu Leu Glu Glu Asp Pro Thr Asp Glu Pro Leu Asn
 225 230 235 240
 Leu Ser Leu Lys Arg Pro Lys Cys Ser
 245

<210> 54
 <211> 233
 <212> PRT

<213> Human adenovirus type 52
 <400> 54

Met Arg Leu Val Pro Glu Met Tyr Gly Val Phe Cys Ser Glu Thr Val
 1 5 10 15
 Arg Asn Ser Asp Glu Leu Leu Asn Thr Asp Leu Leu Asp Val Pro Asn
 20 25 30
 Ser Pro Val Thr Ser Pro Pro Ser Leu His Asp Leu Phe Asp Val Glu
 35 40 45
 Val Asp Pro Pro Gln Asp Pro Asn Glu Asp Ala Val Asn Ser Met Phe
 50 55 60

Pro Glu Cys Leu Phe Glu Ala Ala Glu Glu Gly Ser His Ser Ser Glu
 65 70 75 80
 Glu Ser Lys Arg Gly Glu Glu Leu Asp Leu Lys Cys Tyr Glu Glu Cys
 85 90 95
 Leu Pro Ser Ser Asp Ser Glu Thr Glu Gln Thr Gly Gly Asp Gly Cys
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 Thr Glu Pro Val Val Lys Asn Glu Pro Val Leu Asp Arg Pro Asp Gln
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Pro Gly His Gly Cys Arg Ala Cys Ala Phe His Arg Asn Ala Ser Gly
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 Asn Pro Glu Thr Leu Cys Ala Leu Cys Tyr Leu Arg Leu Thr Ser Asp
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 Phe Val Tyr Ser Asp Val Ser Asp Ala Glu Gly Asp Gly Asp Arg Ser

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Gly Ser Ala Asn Ser Pro Cys Thr Leu Gly Ala Val Val Pro Val Gly
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Ile Ile Lys Pro Val Ala Val Arg Val Ser Gly Arg Arg Cys Ala Val
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Glu Lys Leu Glu Asp Leu Leu Gln Glu Glu Gln Thr Glu Pro Leu Asp
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Leu Ser Met Lys Arg Pro Lys Leu Thr
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<212> PRT

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20 25 30

Pro Ala Asn Phe Val Ile Pro Pro His Gly Val Val Leu Leu Tyr Leu
35 40 45

His Ile Ala Val Asp Ile Pro Pro Gly Tyr Leu Gly Thr Leu Phe Ser
50 55 60

Leu Cys Asp Met Asn Ala Arg Gly Val Phe Val Gly Ala Glu Thr Leu
65 70 75 80

Tyr Pro Gly Ser Arg Met Glu Leu Ser Val Leu Leu Phe Asn His Ser
85 90 95

Asp Val Phe Cys Asp Val Arg Ala Lys Gln Pro Val Ala Arg Leu Leu
100 105 110

Leu Ser Arg Val Val Phe Pro Pro Val Cys Gln Ala Ser Leu Ile
115 120 125

<210> 56

<211> 125

<212> PRT

<213> Human adenovirus type 7

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Met Ala Asp Glu Ala Leu Tyr Val Tyr Leu Glu Gly Pro Gly Ala Thr

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Pro Phe Thr Leu Tyr Pro Arg Gly Val Ala Leu Leu Tyr Leu Arg Leu			
	35	40	45
Ser Ile Ile Ile Pro Arg Gly Tyr Val Gly Cys Phe Phe Ser Leu Thr			
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Asp Ala Asn Met Ser Gly Leu Tyr Ala Ser Ser Arg Ile Ile His Ala			

65	70	75	80
Gly His Arg Glu Glu Leu Ser Val Leu Leu Phe Asn His Asp Asp Arg			
	85	90	95
Phe Tyr Glu Gly Arg Ala Gly Asp Pro Val Ala Cys Leu Val Met Glu			
	100	105	110
Arg Leu Ile Tyr Pro Pro Val Arg Gln Ala Thr Met Ile			
	115	120	125

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<211> 128

<212> PRT

<213> Human adenovirus type 2

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400> 57

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	20	25	30
Ser Pro Ile Asn Phe Val Ile Pro Pro Met Gly Ala Val Met Leu Ser			
	35	40	45
Leu Arg Leu Arg Val Cys Ile Pro Pro Gly Tyr Phe Gly Arg Phe Leu			

50

55

60

Ala Leu Thr Asp Val Asn Gln Pro Asp Val Phe Thr Glu Ser Tyr Ile

65 70 75 80

Met Thr Pro Asp Met Thr Glu Glu Leu Ser Val Val Leu Phe Asn His

85 90 95

Gly Asp Gln Phe Phe Tyr Gly His Ala Gly Met Ala Val Val Arg Leu

100 105 110

Met Leu Ile Arg Val Val Phe Pro Val Val Arg Gln Ala Ser Asn Val

115 120 125

<210> 58

<211> 128

<212> PRT

<213> Human adenovirus type 5

<400> 58

Met Ala Ala Ala Val Glu Ala Leu Tyr Val Val Leu Glu Arg Glu Gly

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Ala Ile Leu Pro Arg Gln Glu Gly Phe Ser Gly Val Tyr Val Phe Phe

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Ser Pro Ile Asn Phe Val Ile Pro Pro Met Gly Ala Val Met Leu Ser

35 40 45

Leu Arg Leu Arg Val Cys Ile Pro Pro Gly Tyr Phe Gly Arg Phe Leu

50

55

60

Ala Leu Thr Asp Val Asn Gln Pro Asp Val Phe Thr Glu Ser Tyr Ile

65 70 75 80

Met Thr Pro Asp Met Thr Glu Glu Leu Ser Val Val Leu Phe Asn His

85 90 95

Gly Asp Gln Phe Phe Tyr Gly His Ala Gly Met Ala Val Val Arg Leu

100 105 110

Met Leu Ile Arg Val Val Phe Pro Val Val Arg Gln Ala Ser Asn Val

115 120 125

<210> 59

<211> 125

<212> PRT

<213> Human adenovirus type 9

<400> 59

Met Ala Glu Ser Leu Tyr Ala Phe Ile Asp Ser Pro Gly Gly Ile Ala

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Pro Val Gln Glu Gly Thr Ser Asn Arg Tyr Thr Phe Phe Cys Pro Glu

20 25 30

Ser Phe His Ile Pro Pro His Gly Val Val Leu Leu His Leu Lys Val

35 40 45

Ser Val Leu Val Pro Thr Gly Tyr Gln Gly Arg Phe Met Ala Leu Asn

50 55 60

Asp Tyr His Ala Arg Asp Ile Leu Thr Gln Ser Asp Val Ile Phe Ala

65 70 75 80

Gly Arg Arg Gln Glu Leu Thr Val Leu Leu Phe Asn His Thr Asp Arg

85 90 95

Phe Leu Tyr Val Arg Lys Gly His Pro Val Gly Thr Leu Leu Leu Glu

100 105 110

Arg Val Ile Phe Pro Ser Val Lys Ile Ala Thr Leu Val

115 120 125

<210> 60

<211> 124

<212> PRT

<213> Human adenovirus type 4

<400> 60

Met Asp Ala Gln Val Leu Tyr Val Phe Leu Glu Gly Ala Gly Ala Leu

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Leu Pro Val Gln Lys Gly Ser Asn Tyr Ile Phe Tyr Ala Pro Ala Asn

20 25 30

Phe Val Leu His Pro His Gly Val Ala Leu Leu Glu Leu Arg Leu Ser

35 40 45

Ile Val Val Pro Gln Gly Phe Ile Gly Arg Phe Phe Ser Leu Thr Asp

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Ala Asn Val Pro Gly Val Tyr Ala Ser Ser Arg Ile Ile His Ala Gly
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His Arg Glu Gly Leu Ser Val Met Leu Phe Asn His Asn Val Ser Phe
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Tyr Asn Gly Arg Ala Gly Asp Pro Val Ala Cys Leu Val Leu Glu Arg

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Val Ile Tyr Pro Pro Val Arg Gln Ala Ser Met Val

115 120

<210> 61

<211> 126

<212> PRT

<213> Human adenovirus type 52

<400> 61

Met Ala Asp Gln His Ile Tyr Val His Leu Leu Gly Arg Arg Ala Phe

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Leu Pro Gln Gln Gln Gly Tyr Ser Asn Met Tyr Val Leu Phe Ser Pro

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Glu Asp Phe Val Leu Ala Pro Arg Gly Ile Ile Leu Leu Ser Leu Gln

35 40 45

Leu Ser Leu Asp Ile Pro Thr Gly Tyr Leu Gly Arg Phe Phe Ser Val

50 55 60

Ala Asp Met Asn Val Arg Gly Val Leu Leu Cys Ala Gln Glu Ile Gln

65 70 75 80

Pro Ser Thr Trp Trp Glu Val Ser Val Val Leu Phe Asn His Ser Asp

85 90 95

Glu Phe Tyr Arg Gly Ser Arg Gly Gln Pro Val Ala Cys Leu Leu Leu

100 105 110

Glu Arg Val Ile Tyr Pro Thr Val Arg Gln Ala Ser Leu Val

115 120 125

<210> 62

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35 40 45

Pro Glu Cys Asn Thr Leu Thr Met His Asn Ala Trp Thr Ser Pro Ser
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Pro Pro Val Glu Gln Pro Gln Val Gly Gln Gln Pro Val Ala Gln Gln
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Leu Asp Ser Asp Met Asn Leu Ser Glu Leu Pro Gly Glu Phe Ile Asn
85 90 95

Ile Thr Asp Glu Arg Leu Ala Arg Gln Glu Thr Val Trp Asn Ile Thr
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Pro Lys Asn Met Ser Val Thr His Asp Met Met Leu Phe Lys Ala Ser
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Thr Arg Ile Trp Asn Ile Pro Ser Cys Tyr Asn Glu Lys Ser Asp Leu

35 40 45

Ser Glu Ala Trp Leu Tyr Ala Ile Ile Ser Val Met Val Phe Cys Ser

50 55 60

Thr Ile Leu Ala Leu Ala Ile Tyr Pro Tyr Leu Asp Ile Gly Trp Asn

65 70 75 80

Ala Ile Asp Ala Met Asn His Pro Thr Phe Pro Ala Pro Ala Met Leu

85 90 95

Pro Leu Gln Gln Val Val Ala Gly Gly Phe Val Pro Ala Asn Gln Pro

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Gly Gly Asp Asp

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