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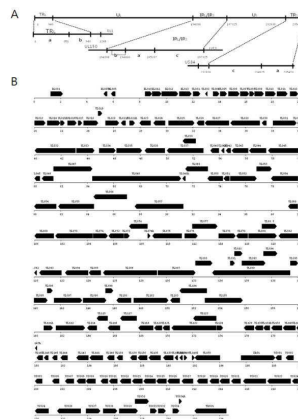
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(54) 발명의 명칭 **한국인 환자로부터 분리된 인간 사이토메갈로바이러스 균주 JHC의 전장 게놈 DNA 및 이의 오픈 리딩 프레임**

(57) 요약

본 발명은 한국인 환자로부터 분리된 인간 사이토메갈로바이러스(human cytomegalovirus, HCMV) JHC 균주 전장 게놈 DNA 및 이의 오픈 리딩 프레임(Open Reading Frame, ORF), 특히 UL1, UL119 및 RL6에 관한 것이다.

대표도 - 도1



특허청구의 범위

청구항 1

서열번호 1의 염기 서열로 이루어지는 인간 사이토메갈로바이러스(human cytomegalovirus, HCMV) JHC 균주 전장 게놈 DNA.

청구항 2

서열번호 2의 염기서열로 이루어지는 UL1, 서열번호 3의 염기서열로 이루어지는 UL119, 및 서열번호 4의 염기서열로 이루어지는 RL6으로부터 선택되는, HCMV JHC 균주 전장 게놈 DNA의 오픈 리딩 프레임(Open Reading Frame, ORF).

청구항 3

제2항에 따른 HCMV JHC 균주 전장 게놈 DNA의 오픈 리딩 프레임에 의해 코딩되는 단백질.

청구항 4

서열번호 1의 염기 서열로 이루어지는 HCMV JHC 균주 전장 게놈 DNA 또는 이의 오픈 리딩 프레임을 포함하는, 형질전환체.

청구항 5

대상 HCMV 균주의 게놈 서열을 결정하고;

상기 결정된 게놈 서열을 서열번호 1의 염기 서열로 이루어지는 HCMV JHC 균주 전장 게놈 DNA 또는 이의 오픈 리딩 프레임과 비교하여 대상 HCMV 균주의 게놈을 분석하는:

단계를 포함하는, HCMV 균주의 게놈 분석 방법.

명세서

기술 분야

[0001] 본 발명은 인간 사이토메갈로바이러스(human cytomegalovirus, 이하 ‘HCMV’ 라 한다)의 전체 게놈 서열분석에 관한 것으로서, 보다 상세하게는, 한국인 환자로부터 분리된 인간 사이토메갈로바이러스 균주 JHC의 전장 게놈 DNA 및 이의 오픈 리딩 프레임(open reading frame, ORF)에 관한 것이다.

배경 기술

[0002] 인간 사이토메갈로바이러스(human cytomegalovirus, 이하 ‘HCMV’ 라고도 칭한다)는 베타-헤르페스바이러스(beta-herpesvirus)에 속하며 인간 바이러스 중에서 가장 큰 230~240 kbp의 이중가닥 DNA를 갖는다. HCMV는 도처에 존재하는 병원체로서 발달된 산업 사회 및 고립된 원주민 그룹에서 전세계적으로 발견된다. 대부분의 HCMV 감염은 증상이 없지만, 신생아, 면역저하된 숙주, 기관 이식의 수혜자에서는 심각한 임상적 결과를 유발할 수 있다.

[0003] HCMV는 1950년대에 최초로 침샘, 아데노이드 조직 및 뇨에서 성공적으로 분리된 이래로 감염 환자의 다양한 부위로부터 분리되었다. 널리 사용되는 실험실 균주 AD169의 게놈이 플라스미드 클로닝과 생거(Sanger) 방법에 의해 전체 서열분석됨으로써 HCMV 유전 정보의 게놈 범위 분석이 가능하게 되었다. 230 Kbp 게놈의 분석 결과 단백질을 암호화할 가능성이 있는 ~150 내지 192개의 오픈 리딩 프레임(open reading frame, ORF)이 밝혀졌다. 대부분의 저계대 바이러스 균주는 고계대 바이러스 균주 AD169에는 존재하지 않는 UL/b' 부위의 ~15 kbp를 포함하는 19개의 ORF를 갖는다. 이 부위는 Ad169의 상응하는 부위와 비교하여 게놈 내에서 역위되어(inverted) 있다.

[0004] HCMV의 전체 게놈 서열을 얻는 것은 HCMV의 큰 게놈 크기로 인해 어려움을 겪어 왔다. 근래에 서열분석 기술들이 개발됨에 따라 HCMV에 대해 거의 20개의 전체 게놈 서열이 결정되었다. 전체 HCMV 게놈의 박테리아 인공 염

색체로의 클로닝은 Toledo, Towne, TB40/E와 같은 잘 알려진 균주와 임상 분리주 FIX, PH 및 TR의 전체 게놈 서열분석을 가능하게 하였다. 최근에 일루미나 게놈 애널라이저(Illumina Genome Analyzer)를 이용한 초고속 서열 분석 기술을 사용하여 Towne 및 AD169 변이체와 몇몇 임상 분리주의 완전한 게놈 서열을 얻었다. 임상 분리주의 전체 게놈 서열을 결정하는데 PCR(polymerase chain reaction) 서열분석 기술이 적용되었다.

[0005] 지금까지 모든 HCMV 분리주의 전체 게놈 서열은 유럽인이나 미국인으로부터 얻어진 것으로서, 아시아인, 특히 한국인으로부터 분리된 HCMV의 전체 게놈 서열은 전혀 보고된 바 없었다.

발명의 내용

해결하려는 과제

[0006] 본 발명자들은 한국인 환자로부터 분리된 HCMV 균주 JHC의 전체 게놈 서열을 밝혀내고, 이에 대한 ORF 분석 및 계통발생학적 분석을 수행하여, 본 발명을 완성하기에 이르렀다.

[0007] 본 발명의 제1 목적은 HCMV JHC 균주의 전장 게놈 DNA를 제공하기 위한 것이다.

[0008] 본 발명의 제2 목적은 HCMV JHC 균주 전장 게놈 DNA의 ORF를 제공하기 위한 것이다.

[0009] 본 발명의 제3 목적은 HCMV JHC 균주 전장 게놈 DNA의 ORF에 의해 코딩되는 단백질을 제공하기 위한 것이다.

[0010] 본 발명의 제4 목적은 HCMV JHC 균주의 전장 게놈 DNA 또는 이의 ORF를 포함하는 형질전환체를 제공하기 위한 것이다.

[0011] 본 발명의 제5 목적은 HCMV JHC 균주의 전장 게놈 DNA 또는 이의 ORF를 이용한 HCMV 균주의 게놈 분석방법을 제공하기 위한 것이다.

과제의 해결 수단

[0012] 본 발명의 제1 면은 서열번호 1의 염기 서열로 이루어지는 HCMV JHC 균주 전장 게놈 DNA에 관한 것이다.

[0013] 본 발명의 제2 면은 상기 HCMV JHC 균주 전장 게놈 DNA의 ORF에 관한 것이다. 구체적인 태양에서, 상기 ORF는 서열번호 2의 염기서열로 이루어지는 UL1, 서열번호 3의 염기서열로 이루어지는 UL119, 및 서열번호 4의 염기서열로 이루어지는 RL6로부터 선택되는 것이다.

[0014] 본 발명의 제3 면은 상기 HCMV JHC 균주 전장 게놈 DNA의 ORF에 의해 코딩되는 단백질에 관한 것이다.

[0015] 본 발명의 제4 면은 상기 HCMV JHC 균주 전장 게놈 DNA 또는 이의 ORF를 포함하는, 형질전환체에 관한 것이다.

[0016] 본 발명의 제5 면은

[0017] 대상 HCMV 균주의 게놈 서열을 결정하고;

[0018] 상기 결정된 게놈 서열을 상기 HCMV JHC 균주 전장 게놈 DNA 또는 이의 ORF와 비교하여 대상 HCMV 균주의 게놈을 분석하는;

[0019] 단계를 포함하는, HCMV 균주의 게놈 분석 방법에 관한 것이다.

발명의 효과

[0020] 본 발명에 따르면, 최초의 아시아인, 즉 미국인이나 유럽인이 아닌 한국인 환자로부터 분리된 JHC 균주의 전체 뉴클레오타이드 서열이 결정되었다. 이는 계통발생학적 및 비교 유전체 연구 등을 통해 HCMV의 분자 특성을 이해하는데 도움을 줄 수 있으며, JHC 균주는 한국인이나 아시아인 HCMV의 참조 균주(reference strain)로 사용될 수 있다.

도면의 간단한 설명

[0021] 도 1의 A는 HCMV JHC 균주의 게놈 지도이고;

도 1의 B는 HCMV JHC 균주의 ORF 지도이며;

도 2의 A는 전체 게놈 뉴클레오타이드에 기초한 HCMV의 계통수이고;

도 2의 B는 공통 뉴클레오타이드 서열에 기초한 HCMV의 계통수이며;

도 3의 A는 JHC의 UL1에서 4 뉴클레오타이드의 결실로 인한 프레임-쉬프트(frame-shift) 돌연변이를 보여주는 도면이고;

도 3의 B는 JHC의 UL119에서 8 뉴클레오타이드의 결실로 인한 프레임-쉬프트 돌연변이를 보여주는 도면이며;

도 4의 A는 HCMV RL6 유전자의 뉴클레오타이드 서열 정렬을 보여주는 도면이고;

도 4의 B는 HCMV UL6A의 뉴클레오타이드 서열에 기초한 네이버-조이닝법을 이용한 계통수를 보여주는 도면이며;

도 4의 C는 HCMV UL6A의 아미노산 서열에 기초한 네이버-조이닝법을 이용한 계통수를 보여주는 도면이다.

발명을 실시하기 위한 구체적인 내용

- [0022] 본 명세서에 사용된 용어 ‘오픈 리딩 프레임’ 또는 ORF는 아미노산 서열로 번역되는 DNA 염기서열로서, 번역 개시 코돈(예: ATG)에서 종결 코돈(예: TGA, TAA, TAG)에 이르는 염기서열을 가리키는 것이다.
- [0023] 본 명세서에 사용된 용어 ‘형질전환체’ (transformant)는 외래 유전자의 획득에 의해 유전적으로 변화된 세포를 가리키는 것으로서, 예를 들어 형질전환된 미생물, 특히 세균, 예를 들어 대장균(*E. coli*)을 포함하는 것이다.
- [0024] 본 발명은 한국인 환자로부터 분리된 JHC 균주의 전체 게놈 서열분석에 관한 것이다. 상기 바이러스는 간시클로비르(gancyclovir) 치료에 대해 내성을 나타내는 골수 이식 환자로부터 분리되었다. 그러나 분리된 바이러스는 간시클로비르 감수성인 것으로 밝혀졌다. 이것은 세포 배양에서 증식에서 잇점을 갖는 소수 바이러스 종이 선별되었기 때문으로 보인다. 이 바이러스는 JHC로 명명되었으며, 본 발명에서는 이 바이러스 균주의 전체 게놈 서열을 결정하였다. JHC 균주는 전체 게놈 서열이 결정된 최초의 아시아인 균주이다.
- [0025] JHC 균주의 게놈 구조는 반복 서열로 둘러싸인 특유의 긴(U1) 부위와 특유의 짧은(Us) 부위로 이루어지는, HCMV의 전형적인 것이다. U1 부위의 3' 말단에 JHC 균주는 U1/b' 으로 알려진 19개의 ORF를 가지며, 이것은 고도로 실험실 적응된 AD169에는 존재하지 않는 것이다. 제한된 시험관 내 세포 배양 계대를 거친 모든 임상 분리주는 U1/b' 서열을 가지며 JHC 균주도 예외가 아니다.
- [0026] 서열분석 결과 JHC 균주의 대부분의 ORF는 다른 균주의 것들과 일치하는 것으로 밝혀졌다. 예외는 초기 종결이 일어나는 두 ORF(UL1 및 UL119)와 비정상적인 개시 코돈을 갖는 하나의 ORF(RL6)이다. 초기 종결은 보통 3n+1 or 3n-1 뉴클레오타이드의 삽입 또는 결실로 인한 프레임-쉬프트 돌연변이에 의해 유발된다. JHC 균주의 경우, 4 또는 8 뉴클레오타이드의 결실로 인해 프레임-쉬프트 돌연변이가 발생한다. 이들 ORF는 모두 HCMV 복제에 필수적이지 않으며 초기 번역 종결은 바이러스 복제에 큰 해를 끼치지 않는다. RL6는 RL11 웨일리의 하나로 본 발명에 사용된 12 HCMV 균주들 사이에서 164개의 ORF중 가장 가변적인 것으로, JHC 균주는 비정상적인 개시 코돈 TTG를 갖는다.
- [0027] JHC는 14개의 마이크로RNA(microRNA, miRNA)에 대한 모든 유전 정보를 가지고 있으며, miRNA 서열은 다른 HCMV 균주에서와 같이 거의 100% 보존되어 있다. 유일한 예외는 첫 번째 염기 T가 C로 치환된 miR-UL70-1-5p의 서열로, 이 치환은 Merlin, HAN13, HAN20 및 3157에서도 발견된다.
- [0028] 최근 연구는 시험관 내 세포 배양에 대한 적응 동안 돌연변이의 축적을 시사한다. 가장 광범위하게 영향을 받는 유전자는 RL13과 유전자 UL128, UL130 및 UL131A로 이루어지는 UL128 좌위(UL128L)이다.
- [0029] 이하, 본 발명을 실시예에 의해 보다 구체적으로 설명하나, 이는 본 발명의 이해를 돕기 위한 것일 뿐 본 발명의 범위를 어떤 식으로든지 제한하고자 하는 것은 아니다.

[0030] 실시예 1: 바이러스 및 DNA 시퀀싱

- [0031] HCMV 균주 JHC를 골수 이식을 받은 한국인 환자로부터 분리하였다(Jung GS, Kim YY, Kim JI, Ji GY, Jeon JS, Yoon HW, Lee GC, Ahn JH, Lee KM, Lee CH., 2011, Full genome sequencing and analysis of human cytomegalovirus strain JHC isolated from a Korean patient., Virus Res. Mar;156(1-2):113-20. Epub Jan 19.). 환자는 간시클로비르 치료에 대해 내성을 나타내었으나, 분리된 바이러스는 간시클로비르 감수성으로서 UL97 유전자에 M460V 돌연변이를 갖지 않았다. 환자 혈액으로부터의 바이러스를 인간 포피 섬유아세포(Human

Foreskin Fibroblast(HFF) cell)로 접종하여 플라스크를 정제하고 HFF 세포에서 3 계대를 추가로 수행하였다. 서열분석에 사용된 바이러스는 4 계대였다.

[0032] DNA를 QIAamp DNA 미니 키트(QIAGEN)로 JHC 스타크로부터 5 μ g/100 μ l의 농도로 추출하였다. DNA 서열을 고속 대량 서열분석법인 마크로젠(MacroGen)에 의해 서비스되는 로슈 다이아그노스틱스(Roche Diagnostics)의 게놈 시퀀서 FLX 스탠다드 시스템(Genome Sequencer FLX Standard System)에 의해 결정하였다. 평균 길이 ~250 bp의 77,390 서열 단편을 얻었고 이들을 Consed 프로그램(<http://bozeman.mbt.washington.edu/consed/consed.html>)을 이용하여 조합 및 가시화하였다. 서열 단편의 평균 퀄리티는 99.99%였다. 총 98.26%의 231,387 서열이 도출된 컨센서스 서열과 일치하였으며 포함 범위는 뉴클레오타이드 당 154 리드(reads)였다. 이들을 두 개의 참조 균주 AD169-varUK(NC_001347.6) 및 Merlin(NC_006273.2)에 대해 정렬하였으며 4개의 큰 콘택(contig)을 얻었다. 콘택들 사이의 공백은 인접한 콘택들로부터 수득된 프라이머를 이용한 PCR 시퀀싱에 의해 채웠다.

[0033] JHC 균주의 완성된 전체 게놈 서열을 서열번호 1에 나타내었다. 서열번호 1에 나타낸 바와 같이, JHC 균주의 게놈은 235,476 bp 길이인 것으로 확인되었다.

[0034] 실시예 2: 오픈 리딩 프레임 분석

[0035] 두 개의 참조 균주 AD169-varUK 및 Merlin에 대한 블라스트 서치(Blast search)에 의해 전체 게놈 서열에서 JHC 균주의 오픈 리딩 프레임(ORF)의 위치를 찾았다. 결과 데이터는 JHC 균주 게놈에 있는 각 ORF의 첫 번째와 마지막 뉴클레오타이드 위치와 ORF의 방향을 포함하는 것이었다. ORF 정보를 CLC 시퀀스 뷰어(CLC Sequence Viewer)(version 6.1 <http://www.clcbio.com/index.php>) 및 NCBI에 의해 제공되는 ORF 파인더(ORF Finder)와 같은 ORF 확인 프로그램에 의해 검증하였다. 블라스트 서치 결과가 ORF 확인 프로그램의 결과와 일치하지 않는 경우, 해당 ORF의 뉴클레오타이드 서열을 바이오에디트 시퀀스 얼라인먼트 에디터(BioEdit Sequence Alignment Editor)(Department of Microbiology, North Carolina State University, version 5.0.9 <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>)로 조사하고 수작업으로 편집하여 개시 및 종결 코돈의 위치를 찾아내었다. 최종적으로 모든 배치된 ORF를 번역된 아미노산 서열의 확인에 의해 확인하였다.

[0036] 상기 분석 결과를 아래 (1) 내지 (4)의 항목에 나타내었다.

[0037] (1) JHC 게놈 구조 및 ORF 맵핑

[0038] JHC 게놈의 구조는 게놈이 6 부위, TRL, UL, IRL, IRS, US 및 TRS로 나누어질 수 있다는 점에서 HCMV의 전형적인 것이었으며, 각 부위의 길이는 각각 940 bp, 193,457 bp, 940 bp, 2,639 bp, 35,512 bp 및 2,639 bp였다. JHC 균주의 게놈 지도를 도 1의 A에 나타내었다. 총 게놈 길이와 구조는 AD169-varUK 균주가 TRL 및 IRL/IRS 부위에 중복된 RL 부위의 존재로 인해 임상 분리주에 비해 훨씬 긴 TRL 및 IRL/IRS 부위를 포함하는 점을 제외하고는 HCMV 균주들과 매우 유사하다. JHC 게놈의 G+C 함량은 분석된 다른 HCMV 균주들과 유사한 대략 57.5%이다. 하기 표 1에 본 발명에서 분석된 HCMV 균주 및 이들의 젠뱅크(Genbank) 등록번호, 게놈 길이 및 GC 함량을 나타내었다.

[표 1] 본 발명에서 분석된 HCMV 균주 및 이들의 ژ놈크 등록번호, 게놈 길이 및

GC 함량

균주	출처	게놈 길이(bp)	%GC
AD169-varUK	NC_001347.6	230,290	57.2
AD169-varUC	FJ527563.1	231,781	57.6
Towne	FJ616285.1	235,147	57.5
HAN38	GQ396662.1	236,112	57.6
HAN20	GQ396663.1	235,728	57.5
HAN13	GQ221973.1	236,219	57.5
3157	GQ221974.1	235,154	57.5
3301	GQ466044.1	235,703	57.5
JP	GQ221975.1	236,375	57.5
Toledo	GU937742.1	235,398	57.5
Merlin	NC_006273.2	235,646	57.5
JHC	본 발명	235,476	57.5

[0039]

[0040]

JHC 균주는 165개의 ORF를 포함하고 있다. 이들 중 7개는 RL 유전자, 125개는 UL 유전자, 31개는 US 유전자이다. 두 개의 다른 ORF는 IRS와 TRS이다. 다른 임상 분리주와 같이, UL133과 UL150 사이의 19개의 ORF는 JHC 균주의 UL 부위의 3' 말단에 위치해있다. 이 부위는 AD169-varUK 균주에서는 중복된 RL 유전자가 점유하고 있으며 ORF UL141, 142 및 144는 AD169-varUK 균주에는 위치하고 있지 않다. JHC 균주의 165개 ORF중 67개는 정방향으로 98개는 역방향으로 존재한다. ORF의 방향은 본 발명에서 분석된 12개 HCMV 균주에서 100% 보존되어 있다. JHC 균주의 ORF 지도를 도 1의 B에 나타내었다.

[0041]

(2) JHC 균주 ORF의 특성분석

[0042]

ORF 서열은 서로 다른 균주들 간에 다소 다양하지만, ORF 길이는 서로 다른 균주들 간에 비교적 유사하다. 165개 ORF중, 78개가 균주에 관계없이 동일한 길이이다. JHC 균주의 두 개의 ORF는 다른 균주에 비해 3 뉴클레오타이드가 짧거나(UL56) 길어서(UL99) JHC 균주에 특이적인 삽입이나 결실 현상을 시사한다. JHC 균주의 UL56에서 다른 HCMV 균주의 1337-1339 위치에 있는 3개의 뉴클레오타이드 ACA의 결실이 있다. JHC 균주의 UL99에서, 다른 HCMV 균주의 344와 345 사이 위치에 GAA의 삽입이 발견되었다.

[0043]

JHC 균주와 다른 주의 ORF를 비교한 결과 두 개의 조기 종결과 비정상적인 개시 코돈이 밝혀졌다. JHC 균주의 두 UL 유전자는 조기 종결 코돈의 형성으로 인해 말단절단되어(truncated) 있다. UL1에서, 69에서 72 사이 위치에서 4 뉴클레오타이드(컨센서스: TGGC)의 결실은 프레임-쉬프트를 일으켜 코돈 번호 25에서 새로운 종결 코돈 TAG를 생성시켰다(도 3의 A). JHC 균주의 UL119에서, 435와 442 사이 뉴클레오타이드 위치에서 8 뉴클레오타이드(컨센서스: TGATAAAG)의 결실로 인해 프레임-쉬프트 돌연변이가 관찰되어, 뉴클레오타이드 위치 505-507에서 새로운 종결 코돈 TGA를 생성시켰다(도 3의 B).

[0044]

JHC 균주는 비정상적인 개시 코돈을 갖는 ORF를 가지고 있다. RL6는 JHC와 3157 균주에서 TTG로 시작한다. HAN13은 비정상적인 ATA 개시 코돈을 가지며, HAN38 균주는 ACG로 시작된다(도 4의 A). 다른 균주들은 모두 보편적인 ATG 개시 코돈을 갖는다. 도 4의 A에 나타난 서열 정렬 데이터는 HCMV가 RL6 서열 다양성에 기초하여 4 그룹으로 나눌 수 있음을 시사한다. JHC는 3157 균주와 클러스터되어 있고 이 클러스터는 다른 그룹과 구별된다. 뉴클레오타이드(도 4의 B) 및 아미노산 서열(도 4의 C) 모두에서 Towne와 HAN13은 또 다른 클러스터를 형성하고 Ad169-varUK, AD169-varUC, Toledo, Merlin, 3301 및 HAN20은 세 번째 클러스터를 형성한다. HAN38은 나머지와 분리되어 있으며 네 번째 클러스터를 형성한다. JHC와 3157 균주는 뉴클레오타이드 및 아미노산 서열에서 매우 유사하다.

[0045]

(3) 마이크로RNA 분석

[0046] HCMV는 적어도 14개의 miRNA에 대한 유전 정보를 가지고 있는 것으로 알려져 있는바, 이들 miRNA 서열이 다른 균주에서와 같이 보존되어 있는지 여부를 조사하였다. 12개의 HCMV 균주들은 miRNA에 대한 모든 유전 정보를 포함하고 있다. JHC 균주와 두 참조 균주 AD169-varUK 및 Merlin의 게놈 상의 miRNA 서열 위치를 하기 표 2에 나타내었다.

[표 2]

miRNA ^a	성숙 miRNA 서열(5' →3') ^b	게놈 위치 ^c		
		AD169-UK	Merlin	JHC
miR-UL22A-1-5p	UAACUAGCCUUCcGUGAgA (서열번호 5)	27,650 - 27,669	27,992 - 28,011	27,753 - 27,772
miR-UL22A-1-3p	UCACcAGAAuGCUaGUUguAG (서열번호 6)	27,687 - 27,708	28,029 - 28,050	27,790 - 27,811
miR-UL36-1	uCGUUGaAGACACCUGGAAAGA (서열번호 7)	49,564 - 49,543	49,914 - 49,893	49,674 - 49,653
miR-UL70-1-5p	uGCGUCUCGGCCUCGUCCAGa (서열번호 8)	104,021 - 104,041	104,405 - 104,424	104,157 - 104,176
miR-UL70-1-3p	GGGGAUggGCUgCGCGCGG (서열번호 9)	104,062 - 104,081	104,445 - 104,464	104,197 - 104,216
miR-UL112-1	AAGUGACGGUGAGAUCCAGGCU (서열번호 10)	164,162 - 164,183	164,557 - 164,578	164,280 - 164,301
miR-UL148D-1	UCGUCCUCCCCUUCUACCCG (서열번호 11)	존재하지 않음	193,587 - 193,607	193,370 - 193,390
miR-US4-1	CGACAUGGACGUGCAGGGGGAU (서열번호 12)	196,090 - 196,111	201,371 - 201,392	201,098 - 201,119
miR-US5-1	UGAcAAGCCUGACGAGAgcGU (서열번호 13)	197,036 - 197,056	202,317 - 202,337	202,048 - 202,068
miR-US5-2	UUAUGAUAGGuGUGACGAUGUC (서열번호 14)	197,163 - 197,184	202,444 - 202,465	202,179 - 202,200
miR-US25-1	AACCGCUCAGUGGCUCCGACC (서열번호 15)	216,245 - 216,225	221,539 - 221,519	221,271 - 221,251
miR-US25-2-5p	Agcggucugucaggugga (서열번호 16)	216,466 - 216,445	221,760 - 221,739	221,492 - 221,471
miR-US25-2-3p	Auccacuuggagagcuccegc (서열번호 17)	216,408 - 216,388	221,702 - 221,682	221,434 - 221,414
miR-US33-1	GAUUGUGCCCGACCGUGGGCG (서열번호 18)	221,468 - 221,447	226,768 - 226,747	226,500 - 226,479

^amiRNA는 Dolken et al.(2009)에 따라 명명.

^b인간과 침팬지 CMV에서 보존되지 않은 뉴클레오타이드는 소문자로 나타냄.

^c게놈 상에 역방향으로 위치한 miRNA 서열에는 밑줄을 친.

[0047]

[0048] 5개의 miRNA(miR-UL36-1, miR-US25-1, miR-US25-2-5p, miR-US25-2-3p 및 miR-US33-1) 서열은 역방향으로 존재한다. miRNA 서열은 JHC 균주와 다른 HCMV 게놈에서 거의 100% 보존되어 있다. 예외는 miR-UL70-1-5p와 miR-UL148D-1이다. AD169-varUK는 UL133과 UL150 사이의 부위가 결실되고 RL 서열에 의해 치환되어 있으므로 miR-UL148D-1을 포함하지 않는다(상기 표 2). JHC에서 miR-UL70-1-5p의 서열은 5' -CGCGTCTCGGCCTCGTCCAGA-3' (서열번호 19)이다. JHC 주에서 첫 번째 염기 T는 C로 치환되어 있으며, 이 치환은 Merlin, HAN13, HAN20 및 3157에서도 발견된다.

[0049] (4) 세포 배양 계대와 관련된 돌연변이

[0050] RL13 및 UL128 좌위(UL128L: UL128, UL130 및 UL131A) 유전자에서의 특정 돌연변이가 HCMV가 섬유아세포에서 성장하는 것을 허용하기 위하여 필요한 것으로 알려져 있다. 이에, JHC 균주와 다른 HCMV 균주에서 이들 유전자에서의 돌연변이가 일어났는지 여부를 조사하였다. 그 결과를 하기 표 3에 나타내었다.

[표 3] 시험관 내 세포 배양에 대한 적응과 관련된 돌연변이

균주	계대 이력 ^a	RL13		UL128		UL130		UL131A		RL6의 비통상적 개시 코돈
		길이 ^b	돌연변이	길이	돌연변이	길이	돌연변이	길이	돌연변이	
AD169-UK	여러 회	147	프레임 쉬프트	171	-	214	-	74	프레임 쉬프트	-
AD169-UC	여러 회	147	프레임 쉬프트	171	-	214	-	74	프레임 쉬프트	-
Towne	여러 회	42	프레임 쉬프트	171	-	229	리드-쓰루 ^e	129	-	-
Toledo	여러 회	209	279 bp 결실	100	년센스 ^d	214	-	129	-	-
HAN13	3	294	-	171	-	214	-	129	-	ATA
3157	3	70	프레임 쉬프트	171	-	214	-	129	-	TTG
Merlin	3	293	-	130	년센스	214	-	129	-	-
JHC	3	306	-	171	-	214	-	129	-	TTG
HAN38	2	301	-	171	-	214	-	129	-	ACG
HAN20	2	302	-	171	-	214	-	129	-	-
3301	무계대	307	-	171	-	214	-	129	-	-
JP	무계대	302	-	171	-	214	-	129	-	-

^a젠뱅크 정보에 따름

^b각 단백질에서 예상 아미노산 잔기 수(밑줄은 아미노산의 유의한 손실을 가리킴).

^c삽입 또는 결실로 인한 조기 종결을 일으키는 프레임-쉬프트 돌연변이: AD169-UK 및 AD169-UC의 RL13에서 1 bp의 삽입 및 UL131A에서 1 bp의 삽입, Towne의 RL13에서 1 bp 삽입 및 3157의 RL13에서 2 bp 삽입.

^dUL128 유전자에서 치환으로 인한 년센스 돌연변이: Toledo의 T302A 및 Merlin의 C391T.

^e뉴클레오타이드 위치 610에서 2 T의 삽입으로 인한 리드-쓰루(read-through) 돌연변이.

[0051]

[0052]

상기 표 3에 나타난 바와 같이, 섬유아세포에서 제한된 계대를 한 JHC 균주와 3개의 다른 주(HAN13, HAN20, HAN38)는 RL13 또는 UL128L 유전자에서 유의한 변화를 나타내지 않았다. 한편, 3157 균주의 RL13에서 프레임-쉬프트 돌연변이, 및 Merlin 균주의 UL128에서 C391T 치환으로 인한 년센스 돌연변이로 인해 제한된 계대의 두 균주에서 조기 종결이 관찰되었다. 시험관 내 세포 계대 이력이 없는 두 균주의 경우, RL13 또는 UL128L 유전자에서 유의한 변화가 관찰되지 않았다. 본 발명에서 분석된 네 균주는 많은 시험관 내 세포 계대를 거친 것으로 알려져 있으며, 모두 RL13에서 279 bp의 결실로 인한 프레임-쉬프트 돌연변이 또는 말단절단으로 인한 조기 종결을 나타내었다. Toledo의 UL128에서의 년센스 돌연변이도 관찰되었다. 또한, Towne 균주의 UL130에서 리드-쓰루 돌연변이가 발견되었고, Toledo 균주의 UL128에서 조기 종결이 발생하였다. 두 AD169 균주에서 모두 프레임-쉬프트 돌연변이로 인해 조기 종결이 발견되었다. 더욱이 흥미롭게도 세포 배양에서 제한된 계대를 거친 6 균주 중 4 균주가 RL6에서 비통상적인 개시 코돈을 갖는 것으로 밝혀졌으며, 이것은 많은 계대를 거치거나 계대를 하지 않은 균주에서는 관찰되지 않는 것이다.

[0053]

실시예 3: 계통발생학적 분석

[0054]

12개의 HCMV 균주들의 전체 뉴클레오타이드 서열에 기초하여 네이버-조이닝법을 이용하여 계통수를 제작하였다.

[0055]

JHC 균주의 뉴클레오타이드 서열을 ClustalW(ver 1.83)로 NCBI 젠뱅크 데이터베이스에 등록된 11개의 다른 HCMV 균주의 뉴클레오타이드 서열과 다중 정렬한 후 수작업으로 편집하였다. 결과 출력 파일을 Phylip 패키지(version 3.6, <http://evolution.genetics.washington.edu/phylip.html>)에 포함된 Dnadist 및 네이버 프로그램을 이용하여 계통수의 제작을 위해 사용하였다. 거리 매트릭스를 키무라-2-파라미터(Kimura-2-parameter)에 의해 수득하였다. 클러스터 분석을 네이버-조이닝 방법에 의해 수행하고 결과 트리 파일을 트리뷰(Treeview) 프로그램(version 1.6.6)에 의해 가시화하였다. 계통수의 유의성을 부트스트랩(bootstrap) 분석에 의해 검증하였다.

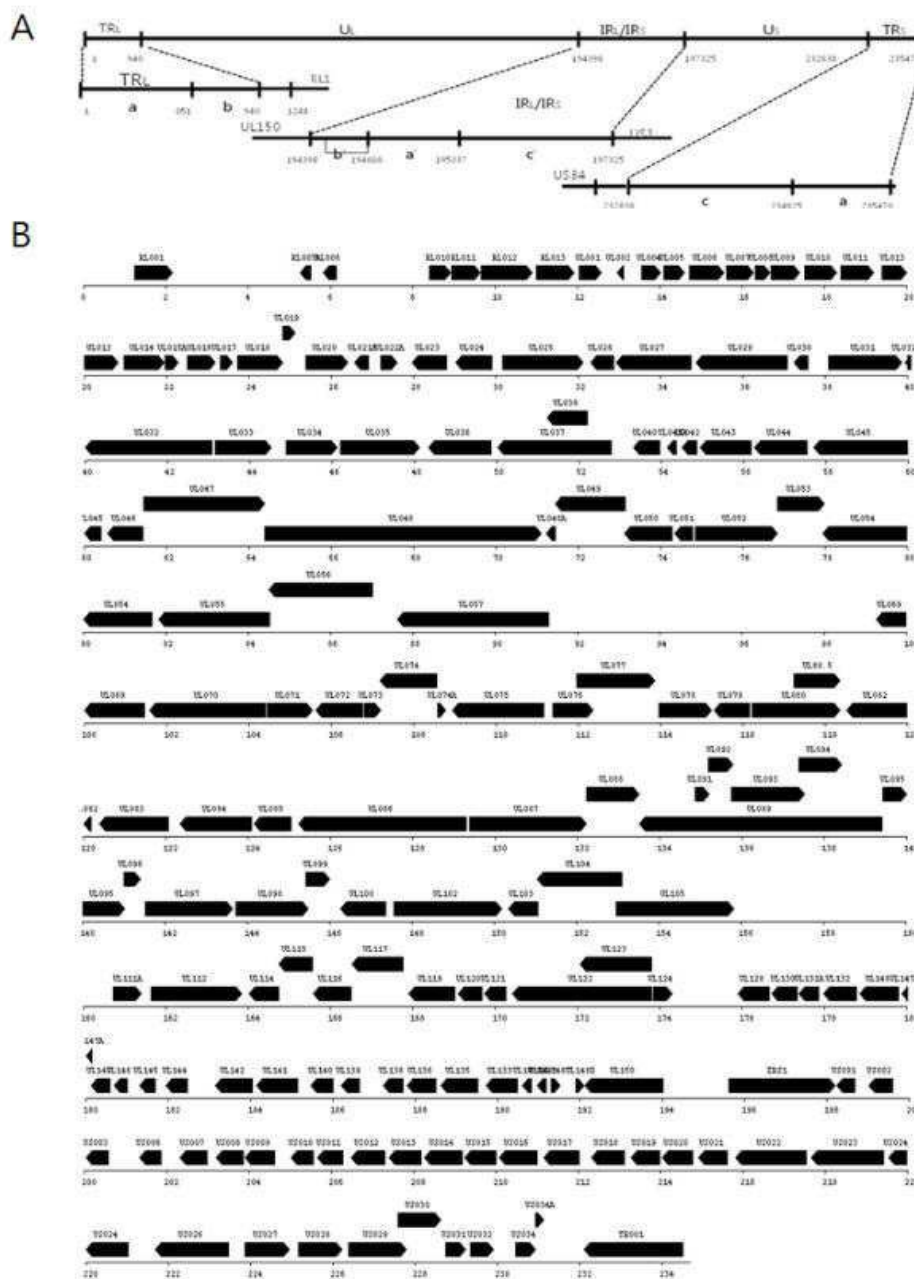
계통수를 Seqboot 프로그램에 의해 생성된 1000개의 레플리케이트(replicates)로부터 구축하고 컨센서스 트리를 컨센스(Consense) 프로그램에 의해 확인하였다.

[0056]

그 결과를 도 2에 나타내었다. 예상과는 반대로, 3개의 실험실 적응주 AD169-varUK, AD169-varUC 및 Towne의 명확한 클러스터링이나 다른 임상 분리주로부터의 분리는 없었다(도 2의 A). JHC 균주는 다른 균주들의 중간에 위치되었다. 실험실 적응주 AD169-varUK는 U1/b' 서열을 갖지 아니하므로, 이들 서열을 전체 계통으로부터 똑같이 제외시키고 계통수를 제작하였다. 12 균주 모두 계통수에서 무작위로 분포하였으며 두 AD169 균주를 제외하고는 어느 특정 균주들의 명확한 클러스터링은 없었다(도 2의 B). 따라서, HCMV 균주들은 적어도 본 발명에서 분석된 12 균주들 사이에서는 전체 뉴클레오타이드 서열에 기초하여 그룹화되지 않는 것으로 보인다.

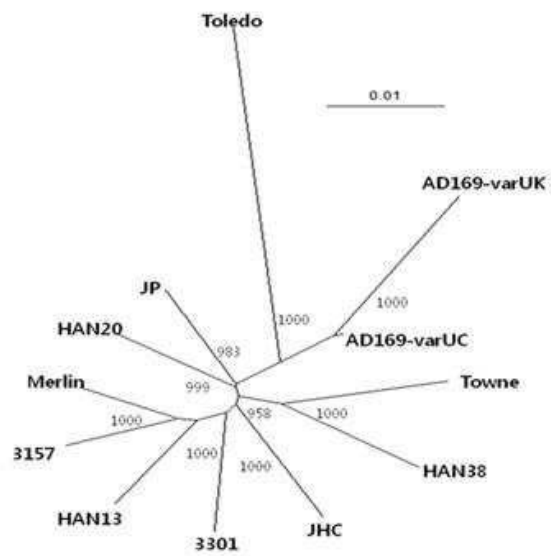
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도면1

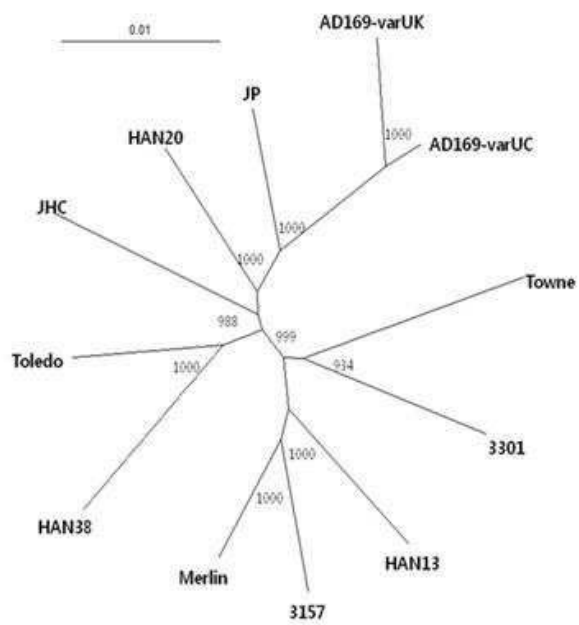


도면2

A



B



도면3

A

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AD169-varUC	ATGSGCATGCAATGCAACACTAAA	ATCCTAATTGGTACTCTAGTG			
Towne	ATGSGTATACAATGTAACACTAAA	ATTCTAAGTACATTTAGTT			
HAN38	ATGSGCATGCAATGCAACACTAAA	ATCCTAATTGGTACTCTAGTG			
HAN20	ATGSGCATGCAATGCAACACTAAA	ATCCTAATTGGTACTCTAGTG			
HAN13	ATGSGTGTACAATATAACACTAAA	ATCCCAAGCTGGCATTCTAGTA			
3157	ATGAGTGTACGATGTAACAGTAAA	ATCCTATCTAGCATCTCTGGTA			
3301	ATGAGCGTACAATGTAACACTAAA	ATCCTAAGTGGAACTCTAGTG			
JP	ATGSGCATGCAATGCAACACTAAA	ATCCTAATTGGTACTCTAGTG			
Toledo	ATGSGCATGCAATGCAACACTAAA	ATCCTAATTGGTACTCTAGTG			
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JHC	ATGSGTGTACAATGTAACAATAAA	ATCCTAAC---ATTCTAGTA			

B

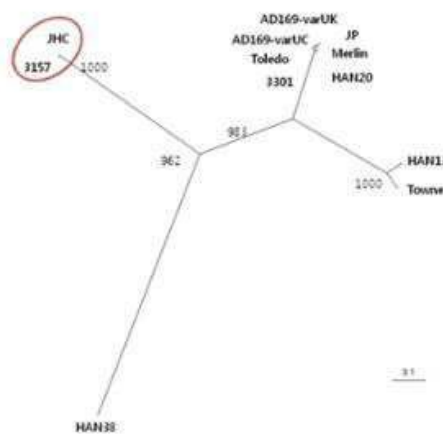
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Towne	ATGTGTCCCGT.	CGGTGATAAAGG.	CGCAGGAGACCACTGAGTA	
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3301	ATGTGCTCCGT.	CAACGATAAAGG.	CGCAGGAGACTACTGAGTA	
JP	ATGTGTCCCGT.	CGGTGATAAAGG.	CGCAGGAGACCACTGAGTA	
Toledo	ATGTGTCCCGC.	CGACGATAAAGG.	CGCAGGAGACCACTGAGTA	
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도면4

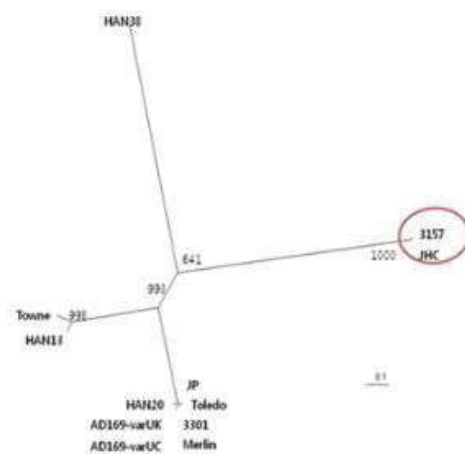
A

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AD169-varUK	ATGACGTTGATATATG	TGGATGCGGATA	
AD169-varUC	ATGACGTTGATATATG	TGGATGCGGATA	
JP	ATGACGTTGATATATG	TGGATGCGGATA	
Toledo	ATGACGTTGATATATG	TGGATGCGGATA	
3301	ATGACGTTGATATATG	TGGATGCGGATA	
Merlin	ATGACGTTGATATATG	TGGATGCGGATA	
HAN20	ATGACGTTGATATATG	TGGATGCGGATA	
3157	TTGACGTTGAATTACA	GAGTCGAGGGAA	
JHC	TTGACGTTGAATTACA	GAGTCGAGGGAA	
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B



C



서열 목록

SEQUENCE LISTING

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