purity of the purified TCR was determined by SDS-PAGE to be greater than 90%, and the concentration thereof was determined by BCA method.

Example 7. Results of BIAcore analysis

The affinity of the αβ heterodimeric TCR, in which a high affinity CDR region was introduced, for FMNKFIYEI-HLA-A0201 complex was detected by using the method described in Example 3.

The CDR regions selected from the high-affinity single-chain TCR α and β chain were transferred into the corresponding positions of the wild-type TCR α chain variable domain SEQ ID NO: 1 and β chain variable domain SEQ ID NO: 2, respectively, to form an αβ heterodimeric TCR. Additionally, αβ heterodimeric TCRs were formed through artificial combination based on the mutation sites screened and obtained in the CDR regions. The amino acid sequences of resulting new TCR α and β chain variable domains are shown in Figures 9(1)-(29) and Figures 10(1)-(20), respectively. Since the CDR regions of a TCR molecule determine their affinity for the corresponding pMHC complex, a skilled person in the art can anticipate that an αβ heterodimeric TCR, in which a high affinity mutation site is introduced also has a high affinity for FMNKFIYEI-HLA-A0201 complex. The expression vector was constructed by the method described in Example 5, the above-mentioned αβ heterodimeric TCR with a high-affinity mutation being introduced was expressed, refolded and purified by the method described in Example 6, and then the affinity of the TCR for FMNKFIYEI-HLA-A0201 complex is determined by BIAcore T200, as shown in Table 4 below.

Table 4

TCR No.	Sequence of α chain variable domain SEQ ID NO:	Sequence of β chain variable domain SEQ ID NO:	K_D(M)
1	54	2	6.3E-07
2	52	2	1.6E-06
3	51	2	6.2E-07
4	53	2	3.5E-07
5	55	2	1.4E-06
6	56	2	9.04E-07
7	57	2	4.65E-07
8	58	2	1.41E-06
9	59	2	2.34E-07
10	60	2	3.99E-07
11	61	2	7.87E-07
12	62	2	5.39E-07
13	63	2	9.99E-07
14	1	78	2.96E-06

Claims

1. A T cell receptor (TCR) having an activity of binding to FMNKFIYEI-HLA A0201 complex, and the T cell receptor comprises a TCR α chain variable domain and a TCR β chain variable domain, the TCR α chain variable domain comprises three CDR regions, and the reference sequences of the three CDR regions of the TCR α chain variable domain are listed as follows,

CDR1α: TSINN

CDR2α: IRSNERE

CDR3α: ATDPRTGANSKLT,

the TCR β chain variable domain comprises three CDR regions, and the reference sequences of the three CDR regions of the TCR β chain variable domain are listed as follows,

CDR1β: SEHNR

CDR2β: FQNEAQ

CDR3β: ASEGLAYEQY,

wherein the TCR α chain variable domain contains at least one of the following mutations:

Residue before mutation	Residue after mutation
S at position 2 of CDR1α	D or P or A or E
I at position 3 of CDR1α	V
N at position 4 of CDR1α	S or Y or D
N at position 5 of CDR1α	S or K
R at position 5 of CDR3α	A or S or V or D or E or Q or T
T at position 6 of CDR3α	M or R or L or A or N or S
G at position 7 of CDR3α	A or Q or S or E or N
A at position 8 of CDR3α	S or G or P or W
N at position 9 of CDR3α	S or T
S at position 10 of CDR3α	A or N or T or P

and/or, the TCR β chain variable domain and contains at least one of the following mutations:

Residue before mutation	Residue after mutation
S at position 1 of CDR1β	A or Q or G
E at position 2 of CDR1β	G or T or R or L
H at position 3 of CDR1β	W or R
N at position 4 of CDR1β	A or P
Q at position 2 of CDR2β	H
N at position 3 of CDR2β	G
E at position 4 of CDR2β	D
A at position 5 of CDR2β	M or L
Q at position 6 of CDR2β	E

and the α chain variable domain of the TCR comprises an amino acid sequence having at least 90% sequence homology with the amino acid sequence shown in SEQ ID NO: 1; and/or the β chain variable domain of the TCR comprises an amino acid sequence having at least 90% sequence homology with the amino acid sequence shown in SEQ ID NO: 2.

2. The TCR of claim 1, wherein there are 3-8 mutations in the 3 CDR regions of the TCR α chain variable domain and/or 4-8 mutations in the 3 CDR regions of the TCR β chain variable domain; or

the affinity of the TCR for FMNKFIYEI-HLA A0201 complex is at least 2 times of that of the wild type TCR; or

the mutated position in the α chain variable domain of the TCR includes position 7 and / or position 8 of CDR3α.

3. The TCR of claim 1, wherein the mutation in the α chain variable domain of the TCR includes the following group:

Residue before mutation	Residue after mutation
G at position 7 of CDR3α	S
A at position 8 of CDR3α	P

and / or the mutation in the β chain variable domain of the TCR includes the following group:

Residue before mutation	Residue after mutation
N at position 3 of CDR2β	G
E at position 4 of CDR2β	D
Q at position 6 of CDR2β	E.

4. The TCR of claim 1, wherein the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and said TCR α chain variable domain comprises CDR1α, CDR2α and CDR3α, wherein the amino acid sequence of CDR1α is selected from the group consisting of: TSINN, TPISS, TDIYK, TAVDS, TEISS and TDVSS; and /or the amino acid sequence of CDR2α is IRSNERE; and / or the CDR3α comprises a sequence selected from the group consisting of: ATDPSMSPNSKLT, ATDPSLSPNSKLT and ATDPSLQSNSKLT.

5. The TCR of claim 1, wherein the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and said TCR β chain variable domain comprises CDR1β, CDR2β and CDR3β, wherein the CDR2β comprises a sequence selected from the group consisting of: FQGDME, FQGDLE and FHGDLE; and / or

the amino acid sequence of CDR3β is ASEGLAYEQY.

6. The TCR of claim 1, wherein the TCR has CDRs selected from the group

consisting of:

CDR No.	CDR1α	CDR2α	CDR3α	CDR1β	CDR2β	CDR3β
1	TSINN	IRSNERE	ATDPSRQSNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
2	TSINN	IRSNERE	ATDPRTGSSNKLT	SEHNR	FQNEAQ	ASEGLAYEQY
3	TSINN	IRSNERE	ATDPRTASSAKLT	SEHNR	FQNEAQ	ASEGLAYEQY
4	TSINN	IRSNERE	ATDPAMQANSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
5	TSINN	IRSNERE	ATDPVLSANSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
6	TSINN	IRSNERE	ATDPRTSGSTKLT	SEHNR	FQNEAQ	ASEGLAYEQY
7	TSINN	IRSNERE	ATDPDRSPNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
8	TSINN	IRSNERE	ATDPRTASTPKLT	SEHNR	FQNEAQ	ASEGLAYEQY
9	TSINN	IRSNERE	ATDPEAQWNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
10	TSINN	IRSNERE	ATDPRTESTTKLT	SEHNR	FQNEAQ	ASEGLAYEQY
11	TSINN	IRSNERE	ATDPSLQSNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
12	TSINN	IRSNERE	ATDPQNSWNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
13	TSINN	IRSNERE	ATDPASNWNSKLT	SEHNR	FQNEAQ	ASEGLAYEQY
14	TSINN	IRSNERE	ATDPRTGANSKLT	SEHNR	FQGDME	ASEGLAYEQY
15	TDVSS	IRSNERE	ATDPAAQWNSKLT	AGHAR	FQGDME	ASEGLAYEQY
16	TPISS	IRSNERE	ATDPAAQWNSKLT	QGHAR	FQGDME	ASEGLAYEQY
17	TPISS	IRSNERE	ATDPATSPNSKLT	GTWPR	FQGDME	ASEGLAYEQY
18	TDIYK	IRSNERE	ATDPRTASSPKLT	GTWPR	FQGDME	ASEGLAYEQY
19	TAVDS	IRSNERE	ATDPSLSPNSKLT	GTWPR	FQGDME	ASEGLAYEQY
20	TAVDS	IRSNERE	ATDPSLSPNSKLT	QGHAR	FQGDME	ASEGLAYEQY
21	TEISS	IRSNERE	ATDPSLSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
22	TAVDS	IRSNERE	ATDPSMSPNSKLT	AGHAR	FQGDLE	ASEGLAYEQY
23	TEISS	IRSNERE	ATDPSMSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
24	TEISS	IRSNERE	ATDPSMSPNSKLT	GTWPR	FQGDLE	ASEGLAYEQY
25	TDVSS	IRSNERE	ATDPSMSPNSKLT	GRWPR	FQGDLE	ASEGLAYEQY
26	TDVSS	IRSNERE	ATDPTMSPNSKLT	GTWPR	FQGDLE	ASEGLAYEQY
27	TAVDS	IRSNERE	ATDPTMSPNSKLT	QGHAR	FQGDLE	ASEGLAYEQY
28	TAVDS	IRSNERE	ATDPSLSPNSKLT	ARHAR	FQGDLE	ASEGLAYEQY
29	TEISS	IRSNERE	ATDPATSPNSKLT	AGHAR	FQGDLE	ASEGLAYEQY
30	TPISS	IRSNERE	ATDPATSPNSKLT	GRWPR	FQGDLE	ASEGLAYEQY
31	TAVDS	IRSNERE	ATDPSLSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
32	TDVSS	IRSNERE	ATDPSMSPNSKLT	GLWPR	FHGDLE	ASEGLAYEQY
33	TPISS	IRSNERE	ATDPSMSPNSKLT	AGHAR	FQGDME	ASEGLAYEQY
34	TDVSS	IRSNERE	ATDPSMSPNSKLT	GTWPR	FQGDME	ASEGLAYEQY
35	TAVDS	IRSNERE	ATDPTMSPNSKLT	QGRAR	FQGDME	ASEGLAYEQY
36	TAVDS	IRSNERE	ATDPTSSWNSKLT	GRWPR	FQGDLE	ASEGLAYEQY.

7. The TCR of claim 1, wherein the TCR is soluble.

8. The TCR of claim 1, wherein the TCR is an αβ heterodimeric TCR or a single chain TCR; and preferably, the TCR comprises α chain constant region sequence TRAC*01 and β chain constant region sequence TRBC1*01 or TRBC2*01.

9. The TCR of claim 8, wherein the TCR comprises (i) all or part of the TCR α chain except for its transmembrane domain, and (ii) all or part of the TCR β chain except for its transmembrane domain, wherein both of (i) and (ii) comprise the variable domain and at least a portion of the constant domain of the TCR chain.

10. The TCR of claim 9, wherein an artificial interchain disulfide bond is contained between the α chain variable region and the β chain constant region of the TCR;

preferably, cysteine residues forming an artificial interchain disulfide bond between the α chain variable region and the β chain constant region of the TCR are substituted for one or more groups of amino acids selected from the following:

Thr48 of TRAC*01 exon 1 and Ser57 of TRBC1*01 or TRBC2*01 exon 1;

Thr45 of TRAC*01 exon 1 and Ser77 of TRBC1*01 or TRBC2*01 exon 1;

Tyr10 of TRAC*01 exon 1 and Ser17 of TRBC1*01 or TRBC2*01 exon 1;

Thr45 of TRAC*01 exon 1 and Asp59 of TRBC1*01 or TRBC2*01 exon 1;

Ser15 of TRAC*01 exon 1 and Glu15 of TRBC1*01 or TRBC2*01 exon 1;

Arg53 of TRAC*01 exon 1 and Ser54 of TRBC1*01 or TRBC2*01 exon 1;

Pro89 of TRAC*01 exon 1 and Ala19 of TRBC1*01 or TRBC2*01 exon 1; and

Tyr10 of TRAC*01 exon 1 and Glu20 of TRBC1*01 or TRBC2*01 exon 1.

11. The TCR of claim 1, wherein the amino acid sequence of the α chain variable domain of the TCR is selected from the group consisting of: SEQ ID NO: 51-77 and 91-92; and / or the amino acid sequence of the β chain variable domain of the TCR is selected from the group consisting of: SEQ ID NO: 78-88 and 93-101.

12. The TCR of claim 1, wherein the TCR is selected from the group consisting of:

TCR No.	Sequence of α chain variable domain SEQ ID NO:	Sequence of β chain variable domain SEQ ID NO:
1	54	2
2	52	2
3	51	2
4	53	2
5	55	2
6	56	2
7	57	2
8	58	2
9	59	2
10	60	2
11	61	2
12	62	2
13	63	2
14	1	78
15	64	79
16	65	80
17	66	81
18	67	81
19	68	81
20	68	80
21	69	79
22	70	82
23	71	79
24	71	83
25	72	84

26	73	83
27	74	85
28	68	86
29	75	82
30	66	84
31	68	79
32	72	87
33	76	79
34	72	81
35	74	88
36	77	84
37	1	93
38	1	94
39	1	95
40	1	96
41	1	97
42	1	98
43	1	99
44	1	100
45	91	101
46	92	101.

13. The TCR of claim 1, wherein the TCR is a single-chain TCR;

preferably, the TCR is a single-chain TCR consisting of an α variable domain and a β variable domain, and the α variable domain and the β variable domain are connected by a flexible short peptide sequence (linker).

14. The TCR of claim 1, wherein the TCR is selected from the group consisting of:

TCR No.	Sequence of α chain variable domain SEQ ID NO:	Sequence of β chain variable domain SEQ ID NO:
s-1	12	4
s-2	10	4
s-3	9	4
s-4	11	4
s-5	13	4
s-6	14	4
s-7	15	4
s-8	16	4
s-9	17	4
s-10	18	4
s-11	19	4
s-12	20	4
s-13	21	4
s-14	3	36
s-15	22	37
s-16	23	38
s-17	24	39
s-18	25	39
s-19	26	39
s-20	26	38
s-21	27	37
s-22	28	40
s-23	29	37

s-24	29	41
s-25	30	42
s-26	31	41
s-27	32	43
s-28	26	44
s-29	33	40
s-30	24	42
s-31	26	37
s-32	30	45
s-33	34	37
s-34	30	39
s-35	32	46
s-36	35	42.

15. The TCR of any one of the preceding claims, wherein a conjugate binds to the α chain and/or β chain of the TCR at C- or N-terminal;

preferably, the conjugate is a detectable label, or a therapeutic agent;

more preferably, the conjugate is an anti-CD3 antibody.

16. A multivalent TCR complex comprising at least two TCR molecules, and at least one TCR molecule is the TCR of any one of the preceding claims.

17. A nucleic acid molecule, comprising a nucleic acid sequence encoding the TCR of any one of claims 1-15, or a complement sequence thereof.

18. A vector, comprising the nucleic acid molecule of claim 17.

19. A host cell, comprising the vector of claim 18 or having the exogenous nucleic acid molecule of claim 17 integrated into its genome.

20. An isolated cell, expressing the TCR of any one of claims 1-15.

21. A pharmaceutical composition, comprising a pharmaceutically acceptable carrier, and the TCR of any one of claims 1-15, or the TCR complex of claim 16, or the cell of claim 20.

22. Use of the TCR of any one of claims 1-15, or the TCR complex of claim 16, or the cell of claim 20 for preparing a medicament for treating tumor; and preferably, the tumor is liver cancer.

23. A method for preparing the T cell receptor of any one of claims 1-15, comprising the steps of:

(i) culturing the host cell of claim 19 to express the T cell receptor of any one of claims 1-15;

(ii) isolating or purifying the T cell receptor.