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TRAV gene expression in PBMCs and TILs in patients with breast cancer analyzed by a DNA melting curve (FQ-PCR) technique for TCR alpha chain CDR3 spectratyping

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Purpose. To explore the expression of the *TRAV* gene in peripheral blood mononuclear cells (PBMCs) and in tumor-infiltrating lymphocytes (TILs) in the patients with breast cancer using a DNA melting curve (FQ-PCR) technique for T cell receptor (TCR) alpha chain CDR3 spectratyping.

Peripheral blood samples and tissue samples were obtained from thirty breast cancer patients. Total RNA was extracted from PBMCs and tumor tissues and then reverse transcribed into cDNA. FQ-PCR was used to amplify the human TCR alpha chain CDR3 region with the primers to the *TRAV* and *TRAC* genes. TCR alpha chain CDR3 spectratyping and partial *CDR3* sequencing were used to determine use of *TRAV* gene product in T cell responses.

TCR alpha CDR3 spectratyping showed preferential usage of certain *TRAV* genes in the PBMCs and TILs of all patients with breast cancer. The frequencies of *TRAV1.1*, *TRAV9*, and *TRAV29* exceeded 30% in PBMCs and the frequencies of *TRAV1.1* and *TRAV22* exceeded 30% in TILs. More than three quarters of the patients (23/30) overexpressed the same gene in both PBMCs and TILs; for example, patient-1 highly expressed *TRAV9* in the PBMCs and TILs. Patients with positive or negative tumor markers of estrogen receptor (ER), progesterone receptor (PR), pS2, C-erbB-2, nm23, P53, and Ki-67 showed no significant common *TRAV* gene expression, but some *TRAV* gene preferential usage frequencies exceeded 20%. For example, five of eight patients positive for ER had high levels of expression of *TRAV1.1* and *TRAV32*. Finally, the amino acid sequence of TCR CDR3 region showed some common motifs in some of the patients.

Conclusions. TRAV gene expression was complex and diverse in the patients with breast cancer. The *TRAV* gene usage may be closely related to the diversity of breast tumor antigens and the differential immune responses observed in individual patients. Research into the immunological mechanism of T cells may provide guidance for individual T cell-directed therapy for breast cancer.

Key words: breast cancer, TRAV gene, TCR CDR3 spectratyping, FQ-PCR, DNA melting curve

Most human mature T cells express alpha or beta T cell receptors (TCRs) for specific recognition of antigenic peptides in the context of major histocompatibility complex (MHC) molecules. Three hypervariable or complementarity-determining regions (CDR1, CDR2, and CDR3) of the TCR have been identified in the alpha and beta chains [1]. The TCR alpha chain gene is rearranged by *TRAV*, *TRAJ*, and *TRAC* gene segments. The region of the TRAV/TRAJ junction corresponds to the TCR alpha chain CDR3. Different sizes or sequences of CDR3 (TCR alpha CDR3 or TCR beta CDR3) correspond to different T cell clones. Therefore, the analysis of the variable region of the TCR CDR3 gene can be used to determine the clonal features of a particular T cell response[2,3].

TCR beta chain and the TCR alpha chain CDR3 spectratyping are of three main types. TCR CDR3 shows preferential usage *TRBV* or *TRAV*; There are common motifs in the CDR3 region or same CDR3 sequences [3, 4]. A correlation between TCR beta chain CDR3 spectratyping and tumor characteristics have been reported for lung cancer [5,6], gastric cancer[7], colorectal cancer[8,9], melanoma[10], glioma [11], and uterine, and ovarian cancers[12]. There appears to be less correlation between TCR alpha-chain CDR3 spectratyping and tumor characteristics[3,13,14] .

In the research of TCR bias and breast cancer, Kirii et al. found that TCR V beta 9 (*TRBV9*), V beta13.1 (*TRBV13.1*), and Vbeta17 (*TRBV17*) were predominantly expressed on specific cytotoxic T cells in the pancreas of breast tumor patients. No preferential expression was found in the TCR V alpha repertoire[15]. Ito et al. reported that there was a predominant TCR V beta 18 family (*TRBV18*) of T cell clonal expansion in the $CD8^+$ T cell subset in the breast cancer patients' lymph nodes, but in most breast cancer patients, peripheral blood mononuclear cells and lymph nodes dominantly express $CD8^+$ T cell clones of the TCR V beta gene families (*TRBV*) [16]. In this study, we observed preferential usage of certain *TRAV* genes in the peripheral blood monocytes (PBMCs) and tumor-infiltrating lymphocytes (TILs) of patients with breast cancer.

Patients and methods

Patients. Peripheral blood samples and tissue samples were collected with informed consent from thirty patients with breast cancer. Peripheral blood was drawn prior to surgical procedures. Tissue samples were obtained from surgical dissections. All the patients were negative for HbsAg, seronegative for markers of hepatitis A, C, D, and E viruses and HIV, and none had clinical or laboratory evidence of other infectious diseases, types of tumors, or immunological disorders. The study protocol was approved by the local ethics committee. Clinical data are summarized in Table 1.

Primers. The primers for *TRAV* family-specific amplification were previously described [17,18]. There were 34 *TRAV* sense primers and a common antisense primer to *TRAC*. Amplification of *GAPDH* (sense: 5'-aggggtctacatggcaact-3'; antisense: 5'-cgaccactttgtcaagctca-3') was used as a control. All the primers were synthesized by the Shang-Hai Invitrogen Corporation of China.

Isolation of mononuclear cells. Five milliliters of peripheral blood were taken from each patient. PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation. Breast cancer tissue was collected and digested by trypase.

Extraction of RNA and synthesis of the first-strand cDNA. Total RNA was extracted from PBMCs and TILs using

Table 1. Clinical information on the thirty patients with breast cancer who took part in this study.

Patients	sex	age	Diagnosis	Samples	ER	PR	PS2	C-erbB-2	nm23	P53	Ki-67
P1	female	29	Invasive ductal carcinoma	PBMC Tissue	+++	-	++	-	+/-	-	15%
P2	female	71	Invasive ductal carcinoma	PBMC Tissue	+++	+	+++	-	+/-	-	<5%
P3	female	62	Invasive ductal carcinoma	PBMC Tissue	-	-	+	++	++	++	30%
P4	female	34	Invasive lobular carcinoma	PBMC Tissue	+	+		+		+	
P5	female	58	Invasive ductal carcinoma	PBMC Tissue	+++	-		-	+		
P6	female	52	Invasive ductal carcinoma	PBMC Tissue	-	-	++	-	+	-	<5%
P7	female	37	Invasive lobular carcinoma	PBMC Tissue	+	-	-	-	++	-	<5%
P8	female	31	Invasive ductal carcinoma	PBMC Tissue	+++	+	++	-		-	5%
P9	male	75	Invasive ductal carcinoma	PBMC Tissue	+	+	+	+	+	-	<5%
P10	female	40	Invasive ductal carcinoma	PBMC Tissue	+	+		+		+	<20%
P11	female	69	Invasive ductal carcinoma	PBMC Tissue	+	-	+	+	+	-	40-50%
P12	female	59	Invasive ductal carcinoma	PBMC Tissue	++	+	++	+	++	+	>30%
P13	female	39	Invasive ductal carcinoma	PBMC Tissue	++	++	+	-	+	+	>50%
P14	female	41	Invasive ductal carcinoma	PBMC Tissue	+	+	+	+		+	<5%
P15	female	61	Invasive ductal carcinoma	PBMC Tissue	-	+		-			
P16	female	37	Invasive ductal carcinoma	PBMC Tissue	+	+	++	++	+		40-50%
P17	female	41	Intraductal carcinoma	PBMC Tissue	+	+	+	+	+	-	10%
P18	female	54	Invasive ductal carcinoma	PBMC Tissue	-	-	-	++		-	10%
P19	female	59	Invasive ductal carcinoma	PBMC Tissue	-	-		-	+	-	
P20	female	64	Invasive ductal carcinoma	PBMC Tissue	++	-	+	-	+	-	5%
P21	female	62	Invasive ductal carcinoma	PBMC Tissue	+	-		++			
P22	female	76	Invasive ductal carcinoma	PBMC Tissue	++	+	+	-	++	+	<20%
P23	female	38	Invasive lobular carcinoma	PBMC Tissue	+++	+++	++	-	-	-	
P24	female	65	Invasive lobular carcinoma	PBMC Tissue	-	-		-		+	50%
P25	female	32	Invasive ductal carcinoma	PBMC Tissue	+++	-	++	+++	+++	+	<5%
P26	female	52	Invasive lobular carcinoma	PBMC Tissue	+	+		_	+	+	30%
P27	female	42	Intraductal carcinoma	PBMC Tissue	+++	-	+++	+++	++	-	10%
P28	female	44	Invasive ductal carcinoma	PBMC Tissue	-	+	+	++	+	+	50%
P29	female	51	Invasive ductal carcinoma	PBMC Tissue	++	++	+	+	+	-	30%
P30	female	52	Invasive ductal carcinoma	PBMC Tissue	+++	+++	+++	++	+++	-	5%

Note: There were no corresponding inspections of tumor markers in part of the patients (P4: PS2& nm23 & Ki-67; P5: PS2& P53 & Ki-67; P8: nm23; P10: PS2& nm23; P14: nm23; P15: PS2& P53& nm23 & Ki-67; P16: P53; P18: nm23; P19: PS2& Ki-67; P21: PS2&P53& nm23 & Ki-67; P23: Ki-67; P24: PS2& nm23; P26: PS2)

the Omega RNA extraction kit according to the manufacturer's instructions. Total RNA was reversely transcribed mixed with 250 pm oligo(dT), 200 U Moloney murine leukemia virus (M-MuLV) reverse transcriptase, and 2 μl of 10 mM dNTP mix (MBI-Fermentas, cDNA synthesis kit) in a total volume of 20 μl (six reactions for every sample). The cDNA was stored at -80 °C before use as a template for PCR amplification.

FQ-PCR amplification of CDR3 cDNA and CDR3 spectratyping. FQ-PCR was carried out in a 20 μl volume with 10 μl 2× Real-time PCR Master Mix (TOYOBO), which contained Taq polymerase, dNTPs, PCR buffers, and SYBR green I. The final concentration of each primer was 0.3 μM, and 1 μl cDNA was added to the reaction mixture as the PCR template. Reactions were performed in a DNA Engine Opticon® 2 System and analyzed with Opticon Monitor 3.0 software (Bio-Rad) using the following procedure: incubation at 94 °C for 3 min, 94 °C melting for 20 sec, primer annealing at 56 °C for 30 sec, and extension at 72 °C for 30 sec for 40 cycles. To ensure an accurate fluorescence signal of the desired amplification products, a high-temperature fluorescence measurement was performed in the fourth step of the PCR run. The fluorescence signal was acquired every cycle at 80 °C for 2 sec. After extension at 72 °C for 10 min, a melting step was performed by slow heating from 75 °C to 95 °C with a ramping of 0.2 °C/sec, during which the fluorescence signal was measured continuously. Amplification reactions for each sample were carried out using the GAPDH specific primer paired with every specific primer for each *TRAV* gene family and melting temperatures (Tms) were determined[9,19,20,21].

Sequencing of CDR3 region. We selected the PCR products of *TRAV* families showing predominant usage, amplified in conditions used for common PCR, and sequenced them on an ABI 377 DNA sequencer (SinoGenoMax Co, Ltd.). DNA tools version 6 was used for analysis[9,21] .

Results

TCR alpha CDR3 spectratyping showed preferential expression of certain *TRAV* genes in the PBMCs and TILs of all 30 patients with breast cancer. The most common *TRAV* genes expressed in PBMCs from these patients (exceeding frequencies of 30%) were *TRAV1.1*, *TRAV9*, and *TRAV29*. In TILs, two genes were expressed by more than 30% of patients:

Table 2. The preferential usage of *TRAV* CDR3 family genes in the PBMCs and tumor tissue of thirty patients with breast cancer.

Patients	TRAV gene preferential usage of PBMCs	TRAV gene preferential usage of TILs	Co-preferential usage
P1	AV9;AV17;AV30;	AV1.1;AV3;AV9;AV13;AV26;AV32;	AV9
P2	AV1.1;AV12;AV17;AV29;	AV1.1;AV17;AV29;	AV1.1; AV17; AV29
P3	AV3;AV13;AV22;AV23;AV29;AV32;	AV1.1;AV6;AV15;AV17	None
P4	AV1.1;AV8;AV9;AV28;	AV1.1;AV9;AV23;AV29;	AV1.1; AV9
P5	AV1.1;AV12;	AV1.1;AV3;AV9;AV12;AV32;	AV1.1; AV12
P6	AV1.1;AV3;AV12;AV21;AV23;	AV1.1;AV8;AV12;	AV1.1 ;AV12;
P7	AV1.1; AV13;AV23;AV26;AV29;	AV1.1;AV12;AV19;AV23	AV1.1; AV23
P8	AV1.1;AV7;AV9;AV12;AV23;AV32;	AV1.1;AV15;AV29;AV32;	AV1.1 ;AV32
P9	AV1.1; AV23;AV32;	AV1.1;AV32;	AV1.1 ;AV32
P10	AV1.1;AV1.2;AV6;AV31;	AV1.1;AV1.2	AV1.1;AV1.2
P11	AV9;AV13;AV28;AV29;	AV8;AV10;AV12;AV29;	AV29;
P12	AV28;	AV22	None
P13	AV9;AV18;AV22;AV28;	AV1.1;AV3;AV22;AV29;	AV22
P14	AV9;AV18	AV22;AV29	None
P15	AV4.2;AV17;AV28;AV32;	AV1.1;AV9;AV22;AV29;	None
P16	AV14;AV19;AV25;AV29;	AV1.1;AV4.2;AV10;AV22;AV32;	None
P17	AV9;AV14	AV10;AV21;AV23;AV25;	None
P18	AV1.1; AV21;AV26;AV29;AV32	AV1.1;	AV1.1
P19	AV9;AV15; AV29;AV32;	AV1.1;AV1.2;AV4.2;AV22;AV32;	AV32
P20	AV1.2 ;AV4.1;AV4.2;AV8;AV23;	AV1.1;AV1.2;AV16;	AV1.2;
P21	AV1.2 ;TRAV4.1;AV9;AV12;AV18;AV25;	AV9;AV12;AV22;AV23;	AV9; AV12
P22	AV10;AV12;AV13;AV22;AV29;AV31;	AV4.1;AV10;AV31;	AV10;AV31;
P23	AV1.2;AV4.1;AV11;AV17;AV19;AV21;	AV1.1;AV1.2;AV2;AV3;AV4.2;AV8;AV19;AV21;AV22AV23;AV27;AV32;	AV1.2; AV19; AV21
P24	AV1.1;AV1.2;AV19;	AV1.2;	AV1.2
P25	AV3;AV18;AV29;	AV3;AV4.1;AV18;	AV3;AV18
P26	AV1.1;AV2; AV7;AV10;AV23;AV29;	AV1.1;AV10;AV22;AV23;AV25;	AV1.1;AV10 ;AV23
P27	AV1.2; AV20;	AV16;AV28;AV32;	None
P28	AV9;AV22;AV25;AV28;	AV12;	None
P29	AV8	AV3;AV8;AV12;AV31;	AV8
P30	AV1.2; AV3;AV5; AV22;AV25;	AV12;AV14;AV22;AV25;	AV22;AV25;

TRAV1.1 and *TRAV22* (Table 2). In 23 of the 30 patients, the same *TRAV* gene was preferentially used in both PBMCs and TILs. For example, *TRAV9* was highly expressed in PBMCs and TILs from patient 1. Data for all 30 patients are summarized in Table 2.

There were no significant correlations between *TRAV* gene preferential usage in TILs and expression of tumor markers including estrogen receptor (ER), progesterone receptor (PR), pS2, C-erbB-2, nm23, P53, and Ki-67. However, some of the *TRAV* gene preferential usage frequency exceeded 20% in patients who expressed certain tumor markers. For example, five of eight patients who had high levels of expression of ER highly expressed *TRAV 32* and *TRAV 1.1* (Table 3).

We observed some common motifs in the amino acid sequences of the TCR CDR3 region (Table 4). For example, *TRAV17* of patient 1 (PBMC), *TRAV4.2* of patient 15 (PBMC), *TRAV3* of patient 16 (PBMC), and *TRAV1.2* of patient 20 (tissue sample) shared the same 'LT' motif. *TRAV4.1* of patient 20 (PBMC) and *TRAV3* of patient 1 (PBMC) both had the 'LI' motif, and *TRAV17* of patient 1 (PBMC) and *TRAV16* of patient 20 (TIL) shared the 'SG' motif. Finally, *TRAV4.1* expressed in PBMCs and *TRAV1.2* expressed in TILs of patient 20 shared the 'GANNL' motif.

Table 3. The *TRAV* gene preferential usage in TILs in patients positive or negative for tumor markers ER, PR, C-erbB-2, P53, and Ki-67

Markers	Results	Patients	The TRAV gene preferential usage	Exceeded 20% of the TRAV gene usage
ER	+++	P1 P2 P5 P8 P23 P25 P27 P30	AV1.1(5/8); AV32(5/8); AV3(4/8); AV9(2/8); AV12(2/8); AV22(2/8); AV29(2/8); AV1.2; AV2; AV4.1; AV4.2; AV8; AV13; AV14; AV15; AV16; AV17; AV18; AV19; AV21; AV23; AV25; AV26; AV27; AV28	AV1.1(5/8); AV32(5/8); AV3(4/8); AV9(2/8); AV12(2/8); AV22(2/8); AV29(2/8)
	-	P3 P6 P15 P18 P19 P24 P28	AV1.1(5/7); AV1.2(2/7); AV12(2/7); AV22(2/7); AV4.2; AV6; AV8; AV9; AV15; AV17; AV29; AV32	AV1.1(5/7); AV1.2(2/7); AV12(2/7); AV22(2/7)
PR	>+	P2 P4 P8 P9 P10 P12 P13 P14 P15 P16 P17 P22 P23 P26 P28 P29 P30	AV1.1(10/17); AV22(8/17); AV29(6/17); AV10(4/17); AV23(4/17); AV32(4/17); AV3(3/17); AV12(3/17); AV25(3/17); AV1.2(2/17); AV4.2(2/17); AV8(2/17); AV9(2/17); AV21(2/17); AV31(2/17); AV2; AV4.1; AV14; AV15; AV17; AV19; AV27	AV1.1(10/17); AV22(8/17); AV29(6/17); AV10(4/17); AV23(4/17); AV32(4/17)
	-	P1P3 P5 P6 P7 P11 P18 P19P20 P21 P24 P25 P27	AV1.1(8/13); AV12(5/13); AV32(4/13); AV1.2(3/13); AV3(3/13); AV9(3/13); AV8(2/13); AV16(2/13); AV22(2/13); AV23(2/13); AV4.1; AV4.2; AV6; AV10; AV13; AV15; AV17; AV18; AV19; AV26 ;AV28; AV29	AV1.1(8/13); AV12(5/13); AV32(4/13); AV1.2(3/13); AV3(3/13); AV9(3/13)
C-erbB-2	>+	P3 P4 P 9 P10 P11 P12 P14P16 P17 P18 P21 P25 P27 P28 P29 P30	AV1.1(6/16); AV12(5/16); AV22(5/16); AV10(3/16); AV23(3/16); AV29(3/16); AV32(3/16); AV3(2/16); AV8(2/16); AV9(2/16); AV25(2/16); AV1.2; AV4.1; AV4.2; AV6; AV14; AV15;AV16; AV17; AV18; AV21; AV28; AV31	AV1.1(6/16); AV12(5/16); AV22(5/16); AV10(3/16); AV23(3/16); AV29(3/16); AV32(3/16)
	-	P1 P2 P5 P6 P7 P8 P13 P15P19 P20 P22 P23 P24 P26	AV1.1(12/14); AV22(5/14); AV32(5/14); AV1.2(4/14); AV3(4/14); AV29(4/14); AV9(3/14); AV12(3/14); AV23(3/14); AV4.2(2/14); AV8(2/14); AV10(2/14); AV19(2/14); AV2; AV4.1; AV13; AV15; AV16; AV17; AV21; AV25; AV26; AV27; AV31	AV1.1(12/14); AV22(5/14); AV32(5/14); AV1.2(4/14); AV3(4/14); AV29(4/14); AV9(3/14); AV12(3/14); AV23(3/14)
P53	>+	P3 P4 P10 P12 P13 P14 P22 P24 P25 P26 P28	AV1.1(5/11); AV22(4/11); AV29(3/11); AV1.2(2/11); AV3(2/11); AV4.1(2/11); AV10(2/11); AV23(2/11); AV6; AV9; AV12; AV15; AV17; AV18; AV25; AV31	AV1.1(5/11); AV22(4/11); AV29(3/11)
	-	P1 P2 P6 P7 P8 P9 P11 P17 P18 P19 P20 P23 P27 P29 P30	AV1.1(10/15); AV32(6/15); AV12(5/15); AV8(4/15); AV1.2(3/15); AV3(3/15); AV22(3/15); AV23(3/15); AV29(3/15); AV4.2(2/15); AV10(2/15); AV16(2/15); AV19(2/15); AV21(2/15); AV25(2/15); AV2; AV9; AV13; AV14; AV15; AV17; AV26; AV27; AV28; AV31	AV1.1(10/15); AV32(6/15); AV12(5/15); AV8(4/15); AV1.2(3/15); AV3(3/15); AV22(3/15); AV23(3/15); AV29(3/15)
Ki-67	≥30%	P3 P11 P12 P13 P16 P24 P26 P28 P29	AV1.1(4/9); AV22(4/9); AV10(3/9); AV12(3/9); AV3(2/9); AV8(2/9); AV29(2/9);AV1.2; AV4.2; AV6; AV15; AV17; AV23; AV25; AV31;AV32	AV1.1(4/9); AV22(4/9); AV10(3/9); AV12(3/9); AV3(2/9); AV8(2/9); AV29(2/9)
	≤5%	P2 P6 P7 P8 P9 P14 P20 P25 P30	AV1.1(6/9); AV12(3/9); AV29(3/9); AV22(2/9); AV32(2/9); AV1.2; AV3; AV4.1; AV8; AV14;AV15; AV16; AV17; AV18; AV19; AV23;AV25	AV1.1(6/9); AV12(3/9); AV29(3/9); AV22(2/9);AV32(2/9)

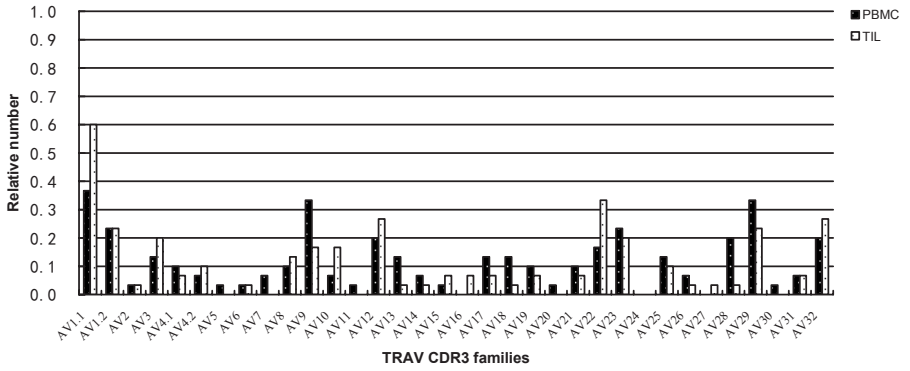

Figure 1. The frequency of the preferential usage of *TRAV* CDR3 families in 30 patients with breast cancer.

Discussion

The CDR3 region of the TCR varies among T cell populations. Specific recognition of a peptide on a certain T cell can induce clonal expansion of T cells that express a particular TCR V gene product. The development of the TCR V gene preferential usage is a sign of an antigen-driven immune response[3,4,22]. Analysis of the CDR3 size and distribution pattern (spectratyping) can be used to define the degree of clonality of the T cells and has been widely exploited in the research of T cell anti-tumor response.

In this study, we used the FQ-PCR technique to determine *TRAV* gene expression in PBMCs and TILs from patients with breast cancer. We found that one or more TRAV families were preferentially expressed in each patient; each of the patients exhibited different *TRAV* profiles (Table 2). In individual patients, *TRAV* gene preference generally differed in PBMCs and TILs. Koichi et al. reported that PBMCs and lymph nodes of most breast cancers patients expressed a dominant $CD8^+$ T cell clone although the number of dominant clones was higher in PBMCs than in the lymph nodes. We did not find the significant different number of the TRAV gene preferential usage between the PBMCs (totals of 119 TRAV gene preferential usage) and TILs (totals of 110 TRAV gene preferential usage) in thirty patients with breast cancer (Table 2) [16]. In the PBMCs of the 30 breast cancer patients we tested, the most commonly expressed *TRAV* genes were *TRAV1.1*, *TRAV9*, and *TRAV29*. In TILs, *TRAV1.1* and *TRAV22* were detected most frequently (Fig. 1). Furthermore, 77% (23/30) of the breast cancer patients had the same *TRAV* gene preferential usage in PBMCs and TILs, and the most highly expressed was *TRAV1.1* (Table 2). Although we did not find a significant correlation

Table 4. The CDR3 gene and protein sequences in parts of patients with breast cancer.

Sample	TCRAV	TCR CDR3 junctional sequence	TCR AJ
P1-PBMC	TRAV17 TGT GAG AGA TCG C E R S	8aa GGA TCA GGA TAC AGC ACC CTC ACC G S G Y S T L T	TRAJ11*01 TTT GGG AAG GGG ACT ATC F G K G T M
P15- PBMC	TRAV4.2 AAA GGA AGC AGC K G S S	5aa CAC TCT AAT CTG ACC H S N L T	TRAJ15*02 TTT GGA AAA GGA ACT CAT F G K G T H
P16-PBMC	TRAV3 TGT GCT ACG GAC C A T D	8aa GCG TGG GCC AAT AGT AAG TTG ACA A W A N S K L T	TRAJ56*01 TTT GGA AAA GGA ATA ACT F G K G I T
P20-PBMC	TRAV 4.1 AGT GCA TCG TCA S A S S	11aa GTC TTG ATC AAA CAT GGG GCA AAC AAC CTC ACC V L I K H G A N N L T	TRAJ29*01 TTT GGG AAA GGA ACT AGA F G K G T R
P1 -TIL	TRAV3 TGT GCT GCG GCG C A A A	8aa GTT GCT GGC GGC GAC AAA CTG ATC V A G G D K L I	TRAJ36*01 TTT GGG ACA GGA ACC AGG F G T G T R
P20-TIL	TRAV1.2 TGT GCT GTG ACC C A V T	8aa CTA ACT GGG GCA AAC AAC CTC TTC L T G A N N L F	TRAJ36*01 TTT GGG ACT GGA ACG AGA F G T G T R
P20-TIL	TRAV16 TGT GCT GTG AGA C A V R	10aa GTC CCA TCA GGA GGA AGC TAC ATA CCT ACA V P S G G S Y I P T	TRAJ6*01 TTT GGA AGA GGA ACC AGC F G R G T S

between *TRAV* gene usage and tumor marker expression, certain frequencies exceeded 20% (Table 3).

All the common TRAV gene preferential usage maybe related to the presence of a certain associated tumor antigen (TAA). Robbins et al. reported that tumor cell antigens in tumor tissue in situ or transferred to the blood circulation are likely to activate the body's immune system[23]. Zhang et al. also reported that T cell clonal proliferation is detected in patients with rectal cancer; these patients had anti-tumor T lymphocytes[24]. Moreover, in some patients with tumors, monoclonal proliferative T lymphocytes activated by tumor antigen are transferred into PBMCs[25]. Other studies reported that minimal residual disease and tumor metastasis results in tumor-associated antigens in the blood circulation and continuously stimulated peripheral blood T lymphocytes. Under certain conditions, clonal proliferation in tumor tissue results in T lymphocytes in the blood. Unconformity with solid tumors may be related to tumor type, level of metastasis, and detection methods[26]. Determination of whether breast cancer-associated antigen is correlated with a high frequency of usage of certain *TRAV* genes will require analysis of larger numbers of patients with more advanced molecular biology techniques than available for this study.

We found that the amino acid sequence of the TCR CDR3 regions in the samples evaluated in our study showed some common motifs (Table 4). Several reports indicate that the CDR3 sequence of antigen-specific T cells that recognize the same peptide have identical CDR3 amino acid motifs[3,27,28]. Whether the motifs we identified are breast cancer-specific must be further affirmed. This type of analysis of gene expression in patient serum and tumor tissue may provide guidance for individualized T cell-directed therapy for breast cancer.

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