Increased expression of activating receptors and up-regulated function of natural killer cells in peripheral blood of patients with HBV-related hepatocellular carcinoma

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Abstract

Background

This study aimed to investigate and identify the characteristics of peripheral natural killer (NK) cells of hepatocellular carcinoma (HCC) patients who infected with hepatitis B virus.

Methods

Flow cytometry was utilized to identify the frequency and receptors of NK cells, in the meantime, to analyze the killing ability and cytotoxicity of NK cells of patients of which embraced 36 cases of HBV-associated HCC, 34 individuals who suffered in HBV-associated cirrhosis (LC) and 30 non-liver dysfunction healthy individuals as control (HC).

Results

Cell counts for NK and $CD56^{dim}$NK were reduced in patients with HCC, however, there was no statistically significant. The count of $CD56^{bright}$NK cells in the HCC subpopulation was remarkably higher than the subset of the HC ($P < 0.05$). The counts of activated receptors of NKG2D/p30 were elevated in patients with HCC, as well as the NKp44 and the NKp46. There was no statistically meaningful difference of inhibitory receptor expressions such as CD158a/b on peripheral NK cells of patients with HCC and LC and those with HC ($P > 0.05$). Following an IL-12 stimulation, the production of INF-γ/-α of patients with HCC was less than those produced by HC ($P < 0.05$). However, killing activity and cytotoxicity, as the primary responsibilities of the natural killer cells, were upregulated in HCC individuals. HBV associated individuals were found lower counts and capability to produce cytokines of natural killer cells. Nevertheless, the killing capacity and cytotoxicity of NK cells were stronger than those of the HC group. This may be associated with the increased activating receptors expression of NK cells.

Conclusions

This study demonstrated that the activating receptors expression and the cytotoxicity of NK cells in the blood were independent predictors of development in HCC patients, and the recovery of $NKG2D^{+}CD56^{dim}$ NK cells could increase the prognostic value.

Background

Based on the date from the WHO (http://gco.iarc.fr/today/data/factsheets/cancers), liver malignancy has been the third most common death cause world widely. Hepatocellular carcinoma (HCC) contributes around 85 percent to 90 percent of the primary liver malignant tumors [1], and about 85 percent of

attributable causes are chronic virial infection such as Hepatitis B (HBV) in China, due to the high prevalence of HBV infection [2]. To date, resection, transcatheter arterial chemoembolization, radiofrequency ablation, stereotactic radiation therapy etc. have been applied widely in the treatments for HCC. However, the outcome of HCC is still unsatisfied since its stubbornly high recurrence rate, which partially caused persistent dysfunction of the antitumor immune response, for example, the dysfunction of immunologic response [3].

Natural killer (NK) cells serve as the primary constituent of the human immunology system. NK cell-mediated innate immunity is maybe crucial in hosting defense against cell malignancy transformation [4, 5]. Since the strongly cytolytic activities of liver-based NK cells have been considered to be essential for HCC immune surveillance [6, 7], functional NK cell capacity probably influences HCC recurrence and prognosis [8, 9]. During the HCC occurrence and development, the distribution and absolute count of NK cells in the liver declined dramatically, which is significantly associated with poor outcomes of liver malignancy. Besides, the capability of NK cell recognition and killing is caused by the balance of activators and inhibitors, which undertake the sensors of pathogens and cancer cells [10, 11].

NK cells express varieties of receptors that transduce inhibitors either activators and identify the specific ligands on codocytes. The dormitory activating receptors, such as CD16 and NKp30/G2D/p46/44 which can recognize the ligands, such as UL-16 protein-ligand family (ULBPs), the major histocompatibility complex (MHC) category I and polypeptide-related sequence A/B to trigger cytotoxic functions and exert antitumor effects of NK cells. In parallel, NKG2A and killer immunoglobulin receptors (KIRs), such as CD158a/b (KIR2DL1/L2/L3, separately), are the primary inhibitory receptors and identify MHC category □ molecules in the majority. Generally, the activated NK cells are mediated by the inhibitors which combined to MHC category □ molecules on their target. In general circumstances, self-cells may sufficiently express MHC category □, which deactivate the NK cells as a result. NK cells may fail to identify the targeted tumor cells since they lack expression of MHC-I, which is a common process of self-recognition loss [10].

It has been known that NK cells utilize strong cytotoxicity against malignant cells, the latter can also regulate the expression of receptors either activation or inhibitors and impair the cytotoxicity function of the natural killer cells. Downregulated expression of activating receptor NKG2D can be related to declined NK cell function and miscarriage recovery of circulating $NKG2D^+$ natural killer cells in HCC after hepatectomy predicts early recurrence [8]. The significantly decreased NKp30 expression demonstrates an escaping mechanism correlated with declined NK cellular activities and cancer development [12]. In addition, these variations are not restricted to activators on NK cells. Some studies had shown that NK cell impairment was associated with upregulated receptors expression such as NKG2A, KIR2DL1/L2/L3 [13–15]. In the liver, NK cells enrich remarkably, where the importance of cytotoxicity and antitumor immunomodulation induced by those cells is broadly recognized. The alterations of the incident and phenotype features of NK cells under the circumstance of HBV-associated HCC are still unclear. This study has identified and demonstrated the different NK cell receptors' expressions and the NK cell killing ratio in HBV-associated HCC individuals, as well as how to influence the activating procedure of NK cells

through the changes of the receptors. This may provide evidence support for the adoptive immunotherapy of HCC.

Natural killer cells can be grouped into different subpopulations according to the expression on the cellular surface markers such as CD16/56. Five NK cell subsets can be identified in human peripheral blood: $CD56^{bright}CD16^{-}$, $CD56^{bright}CD16^{dim}$, $CD56^{dim}CD16^{-}$, $CD^{56dim}CD16^{bright}$, and $CD56^{-}CD16^{bright}$, which were performed according on certain expression of the cellular surface markers CD16 (FcγR III, a cluster of differentiation molecule found on the surface of natural killer cells, as well as low-affinity receptor for the Fc portion of immunoglobulin G) and CD56 [16–18]. In healthy individual's peripheral blood, the $CD56^{dim}CD16^{bright}$NK cells occupy in the majority of 90% in all natural killer cells, followed by the subsets of $CD56^{dim}CD16^{-}$ and $CD56^{-}CD16^{bright}$ [16, 17, 19, 20]. $CD56^{dim}$NK cells largely predominate in peripheral blood (~ 90%) and rapidly demonstrate influential cytolytic activity. However, $CD56^{bright}$ NK cells represent only ~ 10% in peripheral blood but predominate in tissues. These cells are minority cytolytic but efficiently secrete cytokines [21]. Certain cytokines (IL-12/15/18) have been assumed to control the function of NK cells. Upon arousal with these cell factors, the cytotoxicity and the cytokine creation of NK cell subpopulations significantly rise [11]. Therefore, it is crucial to identify peripheral NK cell expression in HBV-related HCC patients.

Despite these associations, the mechanism of the association between NK cells and HBV-associated HCC is not fully revealed. We focused on the roles of the NK cells in antitumor immunologic responses and attempt to identify circulatory natural killer cells in HCC patients with HBV infection, involved with the absolute count, subpopulations, receptors. Subsequently, assess NK cells' immunologic mechanism within the prospective susceptible crowd.

Methods

Study Subjects

Thirty-six HCC patients with HBV infection, 34 HBV-associated cirrhosis (LC), and 30 healthy individuals as control (HC) were recruited consecutively from Shijiazhuang Fifth Hospital during June 2016 and December 2017 and followed up until December 2018. The HCC was diagnosed based on the its standard [22]. HBC objects conformed to the guideline of prophylaxis and treatment for chronic B-type virial hepatitis [23]. All the HCC and LC patients were positive for serum HBsAg and negative for hepatitis C and HIV during the recruitment. Enrolled healthy individuals undertook physical examination to exclude liver diseases and(/or) virus infestation were grouped as HC. Table 1 has listed the clinical and laboratory examinations of all the participants. Peripheral Mononuclear cells (PBMCs) were collected and isolated from all participant individuals. This study was approved by the Ethics Committees of Shijiazhuang Fifth Hospital, Prior to the study, written informed consent was obtained from individual or guardian participants.

Table 1
Clinical and laboratory examinations of all the participants

Variables	**HCC** (n = 36)	**LC** (n = 34)	**HC** (n = 30)	***P* value**
Age (years)	55.75 ± 9.11	47.54 ± 15.29	44.27 ± 13.38	0.0087
Sex, n (%)				
Female	6 (16.67%)	6 (17.65%)	6 (20%)	0.948
Male	30 (83.33%)	28 (82.35%)	24 (80%)	
BMI	22.80 ± 3.26	23.41 ± 2.89	21.56 ± 3.15	0.4556
Blood routine				
RBC (10^{12}/L)	4.12 ± 0.47	4.31 ± 1.25	5.15 ± 1.06	0.3978
WBC (10^9/L)	4.35 ± 2.86	3.81 ± 1.11	7.78 ± 1.56	< 0.001
PLT (10^9/L)	116.55 ± 61.93	105.31 ± 45.23	198.45 ± 54.36	< 0.001
Hb (g/L)	131.35 ± 15.55	135.23 ± 18.35	145.35 ± 20.48	0.4589
HBV DNA (lg IU/mL)	4.61 ± 1.34	5.25 ± 1.54	N.A.	0.0675
Liver function index				
ALT (IU/mL)	34 (13–137)	61 (21–568)	N.A.	0.0059
AST (IU/mL)	37 (18–197)	75 (19–467)	N.A.	0.0042
ALP (IU/mL)	46 (25–751)	35 (23–254)	N.A.	0.2874
GGT (IU/mL)	97.5 (25–328)	78 (28–215)	N.A.	0.0784
CHE (U/L)	4669.70 ± 1447.01	4580.12 ± 1865.56	N.A.	0.8236
AFP (μg/L)	7.6 (1.3-20000)	4.5 (1.5–156)	N.A.	< 0.001
Total protein (g/L)	68.40 ± 6.81	71.54 ± 6.98	N.A.	0.0594
Albumin (g/L)	35.85 ± 4.68	36.31 ± 6.71	N.A.	0.7392
Globulin (g/L)	32.55 ± 5.47	33.64 ± 6.04	N.A.	0.4310
TBil (μmol/L)	23.62 ± 14.70	19.7 1 ± 11.65	N.A.	0.2234

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; AFP: alpha fetal protein; TBil: total bilirubin; DBil: direct bilirubin; PTA: prothrombin time activity; Data are median (minimum-maximum); BMI: body mass index, in kilograms, divided by height squared (in meters); RBC: red blood cells; WBC: white blood cells; PLT: platelet; Hb: hemoglobin; NA: not applicable. P-value refers to a comparison between HCC patients with HC.

Variables	HCC (n = 36)	LC (n = 34)	HC (n = 30)	*P* value
DBil (μmol/L)	10.52 ± 7.53	8.48 ± 5.89	N.A.	0.2154
PTA (%)	82.84 ± 13.42	78.63 ± 17.96	N.A.	0.2687
Child-Pugh classification				
Grade A	24	19	N.A.	0.2480
Grade B	12	15	N.A.	

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; AFP: alpha fetal protein; TBil: total bilirubin; DBil: direct bilirubin; PTA: prothrombin time activity; Data are median (minimum-maximum); BMI: body mass index, in kilograms, divided by height squared (in meters); RBC: red blood cells; WBC: white blood cells; PLT: platelet; Hb: hemoglobin; NA: not applicable. P-value refers to a comparison between HCC patients with HC.

PBMC Isolation And Cell Purification

Mononuclear cells were collected and then isolated by applying the Ficoll-Hypaque centrifugation (Sigma, USA) based on the manufacturer's instruction and stored in liquid nitrogen. Autologous NK cells were thereafter isolated and purified by application of the MACS Natural Killer cell negative selection kit (MACS, Miltenyi Biotec, China), according to the manufacturer's instruction. Flow cytometry was utilized which measured and confirmed the purity of NK cells ($CD3^{-}CD56^{+}$) was higher than 95%.

Frequency And Number Of NK Cell Detection

20 mL fasting venous blood was collected in a heparin anticoagulation tube from each person. The ratio and count of NK cells were detected by the IMK MultiTEST kit (BD Biosciences, USA) and TruCOUNT absolute counter tube and analyzed using FACS MULTISET software (BD Biosciences, USA), in conformity with the manufacturer's instructions.

Flow Cytometry Analysis Of NK Cell Receptors

3 mL PBMCs were prepared to detect NK cell receptors. Flow cytometry was introduced to make detection and measurement. The PBMCs were thawed initially. They were resuspended in complete RPMI1640 medium (Corning Cellgro, USA) which embraced with 10% fetal bovine serum, 1% glutamine and 1% penicillin and streptomycin. 1×10^6 cells were seeded for every panel and treated in the dark to stain at room temperature (20 ~ 25°C) for thirty minutes. NK cell phenotype identification of NK cell was performed via certain monoclonal antibodies which were: FITC conjugated anti-CD158b, PerCP-Cy5.5

conjugated anti-CD56, PB conjugated anti-CD3, BV510 conjugated anti-CD45, APC-Cy7 conjugated anti-CD16, APC conjugated anti-NKG2D/CD158a/NKP44/NKP30 and PE-Cy7 conjugated anti-NKP46. All antibodies were acquired from BD Biosciences (San Jose, CA, USA). The PBMCs have washed twice with PBS fowling staining. A BD LSR II Fortessa flow cytometer (BD Biosciences, San Jose, CA, USA) was utilized for detection and identification. The data were consequently examined via the software FlowJo (TreeStar, Ashland, OR, USA).

Degranulation Of NK Cells And IFN-γ Detection

The cytotoxic potentials of NK cells were assessed by CD107a degranulation. PMA (100 ng/mL, Sigma-Aldrich, USA) and Ionomycin (1 μg/mL, Santa Cruz Biotechnology, USA) were administrated at 37 ºC for 1 h to stimulate the freshly isolated PBMCs (5×10^5 cells/mL). Controls were set with non-stimulated PBMCs. Antibody (APC conjugated anti-CD107a) was administrated to the medium first. GolgiStop was added one hour afterward. Those cells were collected and stained after 5 h of incubation, together with antibodies and cytokine such as PE-conjugated anti-IFN-γ and FITC conjugated anti-TNF-α intracellularly. The detection and measurement were performed by flow cytometry.

Cytolytic Killing Assay

K562 cells were firstly tagged with Carboxy fluorescein succinimidyl ester (CFSE, Molecular Probes, Eugene, OR) which is a fluorescent cell staining dye. PBMCs were subsequently incubated with the CFSE-tagged assay cells for six hours with at ratios of 3:1, 10:1 and 30:1 separately, where the production, as well as the target cells, were set as controls. Those cells were thereafter treated with 1 μg/mL of 7-aminoactinomycin D (BD Biosciences, San Jose, CA, USA) for the verification of apoptosis or necrosis.

Cytokine Stimulation

PBMCs (2×10^6 cells/mL) were cultured along and together with 5 ng/mL of IL-12 for 48 h, separately. Afterward, samples were either stained with antibodies that contrapose to activation markers or further degranulate via IFN-γ and TNF-α to six hours, which release assays in the expression of IL-12.

Statistical analysis

The statistic works were performed by using a statistical software Stata 12.0 (StataCorp LP, Texas, USA). The normal distribution of quantitative data was analyzed by Sktest. The t-test was elected in two-group comparisons of normal distribution data, and the results were expressed as mean ± SEM. Rank sum test was placed for data of two-group comparisons with abnormal distribution, and the results were demonstrated as a median and inter-quartile range. Spearman test was adopted for the rank correlation

in two-group comparisons. A chi-square test was introduced to analyze qualitative data. Statistically significant is defined when two-tailed P values < 0.05.

Results

Low frequency of circulating CD56 ^{dim} NK cells and high frequency of circulating CD56^{bright} NK cells in HCC cases

All the results were shown as median (P25, P75), %. The median of total peripheral NK cells was dwindled in HCC group 18.7 (5.5, 77.3) and LC 16.1 (5.8, 41.2) than in HC 20.5 (6.7, 46.1). Nevertheless, the variation between those three subgroups did not demonstrate a statistically significant (P = 0.121, Fig. 1). Consequently, we compared the subpopulations of NK cells between all three subgroups (Fig. 1). It revealed that the median count of CD56^{dim} cells was dramatically lower than those in LC subset (12.4 (4.3, 34.2)) as compared to HC (16.5 (5.6, 41.8), P = 0.024). No meaningful statistical difference of CD56^{dim} natural kill cells between HCC and LC subpopulations (P = 0.689) has been observed. The median count of CD56^{bright} cells in HCC and LC group was significantly higher than HC (P = 0.006, P = 0.017, respectively) individuals. The difference in CD56^{bright} cells between HCC and LC subgroups did not demonstrate a statistical significance (P = 0.768).

Increased activating receptors expression and declined inhibitory receptors expression in peripheral NK cells of HCC individuals

The activating receptors (NKG2D/p30/p44/p46) expressions and inhibitory receptors (CD158a/b) over all three subgroups were listed in Fig. 2. The inhibitory receptors of CD158a/b were downregulated in HCC conditions, compared to those in HC individuals, which did not demonstrate any statistically significant (P = 0.495, P = 0.633 and P = 0.399, P = 0.154 respectively). The activating receptors expression (NKG2D/p30/p44,/p46) were upregulated on peripheral natural killer cells in subpopulation with HCC (1.1, P = 0.0008; 41.2, P = 0.052; 0.2, P = 0.056; 6.5, P = 0.003; respectively), compared with HC subset (0.4; 32.6; 0.1; 2.4), whereas the expression of NKG2D and NKp30 were upregulated in HCC individuals, compared to condition of LC (P = 0.021, P < 0.001 respectively). However, significant increase of NKp44 (P = 0.005 and NKP46 P = 0.0007) between individuals with LC and HC (Fig. 2) was observed.

Unchanged CD107a degranulation and decreased production of IFN-γ and TNF-α in peripheral NK cells of HCC group

To assess the cytotoxicity of peripheral natural killer cells, we detected their CD107a degranulation and secretion of IFN-γ and TNF-α. The results did not demonstrate a statistical difference of CD107a expression among all the enrolled groups (P = 0.545, HCC vs LC; P = 0.130, LC vs HC; P = 0.341, HCC vs HC) (Fig. 3).

IL-12 was applied to stimulate natural killer cells in all the participant groups. The expression of CD56^{dim} IFN-γ and CD56^{bright} IFN-γ in HCC patients dramatically decreased compared to LC patients and HC (P <

0.001, P < 0.001, P < 0.001, P < 0.001 respectively). However, there was no meaningful statistical difference in the expression of $CD56^{dim}$ IFN-γ in LC and HC groups (P = 0.122). $CD56^{dim}$ TNF-α in LC individuals was significantly decreased compared to HC subgroup (P = 0.0002), while there was no statistical difference between HCC and LC individuals (P = 0.113). The level of $CD56^{bright}$ TNF-α in patients with HCC was significantly declined compared to those in patients with LC (P = 0.030). Nevertheless, no remarkable difference in the LC and HC groups (P = 0.406) (Table 2) has been observed.

Table 2
Frequency of IFN-γ and TNF-α of $CD56^{bright}$ NK and $CD56^{dim}$ NK cells under IL-12 stimulus [median (P25, P75), %]

	$CD56^{dim}$ IFN-γ	$CD56^{bright}$ IFN-γ	$CD56^{dim}$ TNF-α	$CD56^{bright}$ TNF-α
HCC	2.0 (0.2, 32.3)	0.2 (0, 1.9)	17.2 (0.2, 69.4)	0.4 (0, 6.4)
LC	7.4 (4.4, 31.2)	0.4 (0.1, 3.6)	26.9 (2.3, 48.2)	1.3 (0.1, 3.5)
HC	18.8 (1.2, 54.3)	1.0 (0.1, 7.7)	42.2 (8.8, 82.5)	1.0 (0.1, 7.6)
HCC *vs* LC	< 0.001	< 0.001	0.113	0.030
LC *vs* HC	0.122	0.002	0.0002	0.406
HCC *vs* HC	< 0.001	< 0.001	< 0.001	< 0.001

Enhanced Cytolytic Activity Of Nk Cells In Hcc Subpopulation

We revealed the natural killer cells from HCC individuals evoked higher K562 lysis level than those of LC subgroup (P = 0.0001, P = 0.0009, P = 0.0009 respectively) at the effect: target (E: T) ratio of 3:1, 10:1 and 30:1, separately. While the natural killer cells from LC individuals evoked lower K562 lysis level compared to those from HC individuals (P = 0.019, P = 0.012, P = 0.012 respectively) at the 3:1, 10:1 and 30:1 E:T ratio. With the increase of the E:T ratio, the cytolytic activity of natural killer cells enhanced in HCC patients gradually. The result did not express a statistical significant between NK cells in HCC and HC subgroup (P = 0.367, P = 0.279 respectively) at the E:T ratio of 3:1 and 10:1. However, it was observed dramatically increased cytolytic activity of NK cells at the ratio 30:1 (P = 0.016) (Fig. 4).

Discussion

A mountain of previous evidence, particularly from pre-clinical studies of *in vitro* and *in vivo* animal models, suggests that NK cells play functional roles in antitumor defenses. Because they have direct cytotoxic effects against malignant cells and the capability to produce cell factors that activate downstream adaptive responses effectively. Natural killer cells are the first immunologic responders against viral affection. However, a sub-normality of NK cell function may even play an unexpected role in cancer development and promotion. On the basis of the CD56 antigen density, NK cells have been divided into two subsets of CD56-dim and bright. The majority of human natural killer cells (about 90%) are the

dominant expression of CD16 and potent cytolytic activity of $CD56^{dim}$ cells, whereas $CD56^{bright}$ cells that produce a large body of cytokines are about 10%. NK cell receptors mainly include activating receptors NKp30/p44/p46/G2D and KIR2DS, whereas inhibitors as CD158a/b, etc. The change of NK cell receptors is closely related to the cytotoxicity of NK cells, and the development and occurrence of tumors [24]. Downregulated activating receptors expression, as well as upregulated inhibitors expression in peripheral circulation and malignant tissues of HCC patients has been proved by previous investigations [25–27]. However, our study revealed that the activating receptors (NKG2D/p30/p44/p46) expressions were upregulated on NK cells of HCC patients, as compared to those in HC. The results were inconsistent with the above reports. This may be because previous studies generally examined the expression of total NK cell-activating receptors while we detected activating receptors of CD56-dim and bright expressions, respectively [28]. Moreover, a small minority of patients with HCC were treated with TACE or radiotherapy in our study, while TACE and radiotherapy could improve the activating receptors expression [29, 30].

Recent evidence indicates that cytokines indeed promote human NK cell activation. In this study, we used IL-12 as a stimulator to stimulate NK cells to secrete IFN-γ/-α, which can reflect the function of NK cell secreting cytokines. We found that the expression of IFN-γ and TNF-α of $CD56^{bright}$ NK cells and IFN-γ of $CD56^{dim}$ natural killer cells in the HCC sub-population was remarkably decreased than the LC subgroup, consequently, the secreting cytokines function of NK cells was impaired in HCC group [31]. The negative regulation of IFN-γ recognizing human NK cells can inhibit the activating receptor NKG2D expression and enhance the inhibitory receptor NKG2A/B and KIR2DLI expression [32]. Our results demonstrated that the expression of NKG2D/p30 of $CD56^{dim}$ peripheral NK cells of HCC individuals was higher than that in the LC group. This may be linked to the lower IFN-γ expression in HCC group compared with the LC group. In HCC patients, the secretion of IFN-γ decreased by NK cells, which can reduce the inhibiting effect of NKG2D, consequently the expression of NKG2D receptors was elevated.

NK cell features mainly involve antibody-dependent cellular-mediated cytotoxicity, cytolytic granule-mediated cell apoptosis, and cytokine-mediated NK and CTL activation, et al. Our results displayed that the features of NK cells secreted IFN-γ and TNF-α were damaged in peripheral blood. There was no meaningful statistical difference in CD107a between HCC and LC subpopulations, but the expression of NKG2D and NKp30 significantly increased, while the cytotoxicity of K562 cells by NK cells was strengthened. NK cells mainly kill the target via releasing the granzyme and perforin. The E: T ratio of NK cells and killing K562 cells can well reflect its killing capability. NK cells evoke apoptosis of target cells primarily through exocytosis and death receptors. $CD56^{dim}$ NK cells can release perforin to kill the target cells. Our results did not demonstrate a significant statistical in the proportion of $CD56^{dim}$ natural killer cells and degranulated expression of CD107a between the HCC and LC subsets. However, activating receptors expression (NKG2D/p30) in HCC individuals was higher than that in LC subsets. The expression of NKp30 of $CD56^{dim}$ NK cells was rank linked to the cytotoxicity of NK cells at the E:T ratio of 1:10. The characteristics of NK cells killing K562 cells in HCC patients involved in the high activating receptors expression on the $CD56^{dim}$ NK cell surface. The expression of NK cell receptors is closely related to NK cell function. The results showed that NKG2D and NK cell receptors often had a synergistic effect when

NK cells exerted cytotoxic effects. NKG2D can have an antitumor effect when the NK cell receptors were low or no expression. The change of NK cell receptors is highly likely related to the cytotoxicity and the development of malignancies [24].

Conclusions

The present study revealed and identified that the activating receptors expression and the cytotoxicity of natural killer cells were increased in peripheral circulation of HCC individuals, which suggested that its receptors may play critical roles in natural killer cell-induced antitumor immunity. The recovery of $NKG2D^{+}CD56^{dim}$ natural killer cells was linked with the outcome of HCC patients. Our results were well acquainted with the mechanisms of those receptors and HCC development. The frequency of the $NKG2D^{+}CD56^{dim}$ NK subpopulation is highly likely a substantial biomarker for predicting the outcome of individual with HCC who was administrated a hepatectomy. This can provide theoretical support for the selection of appropriate combination immunotherapy by further regulating the cell receptor expression of NK cells.

Abbreviations

NK: natural killer cell; HCC: hepatocellular carcinoma; HBV: hepatitis B virus; ULBPs: UL-16 protein-ligand family; MHC: major histocompatibility complex; KIR: killer immunoglobulin receptor; HC: healthy controls; LC: liver cirrhosis; PBMC: peripheral mononuclear cells.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Shijiazhuang Fifth Hospital. Written informed consent was obtained from individual or guardian participants.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are included within the article.

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

Study concept and design: CXL and JL; Collection and assembly of data: PYT; Performed the experiment: YWF, ZQ and YXY; Data analysis and interpretation: PYT; Manuscript writing and review: PYT, CXL and JL. All authors have read and approved the manuscript in its current state.

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Figures

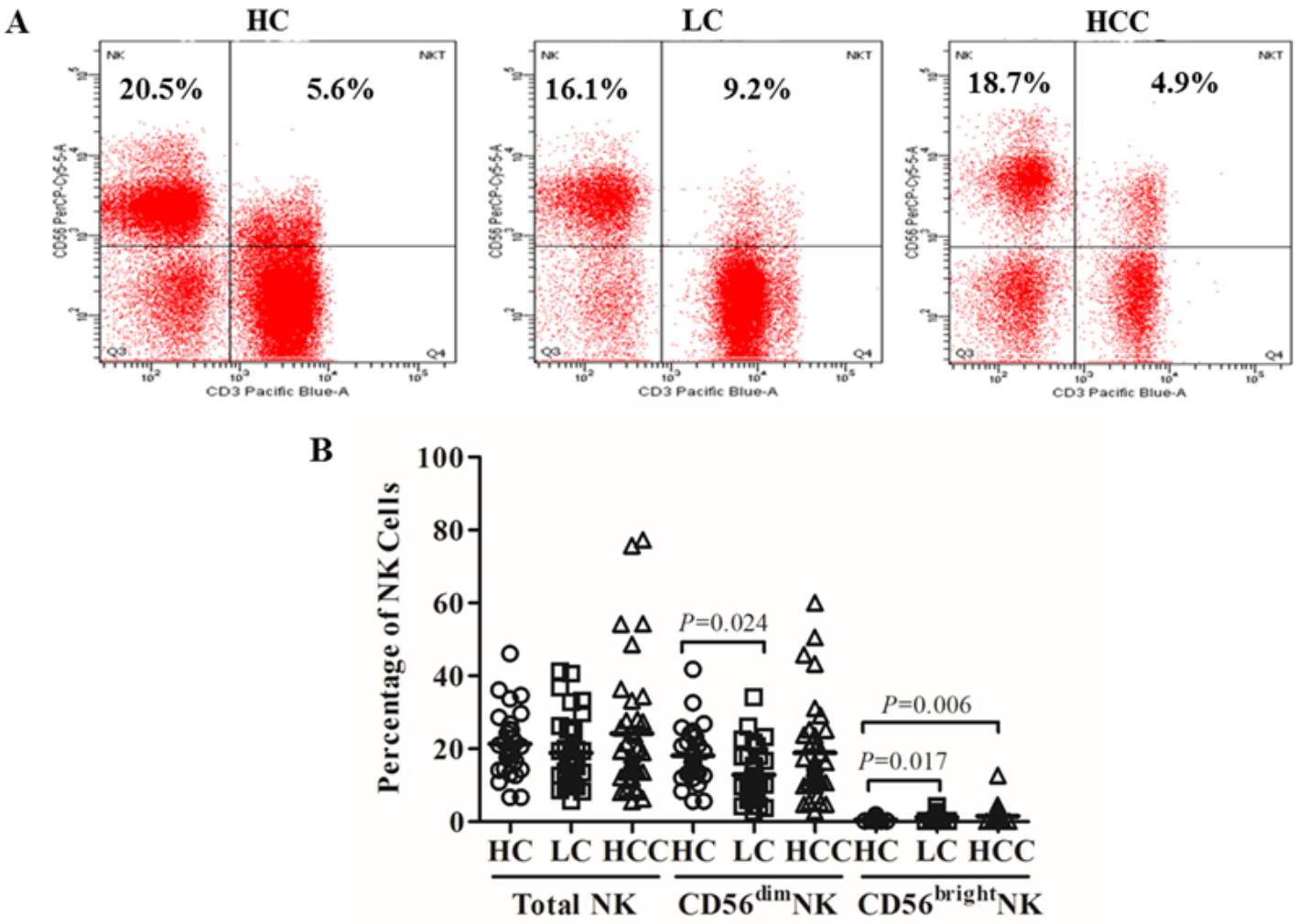

Figure 1

Peripheral NK cells stained in HCC subgroup. (A) demonstrate dot plots of natural killer cell subgroups from HC, LC and HCC individuals according to the certain expression of CD56. (B) Frequencies of natural killer cells contribute lymphocytes of three groups. HC: healthy controls; LC: Liver cirrhosis; liver cancer: HCC.

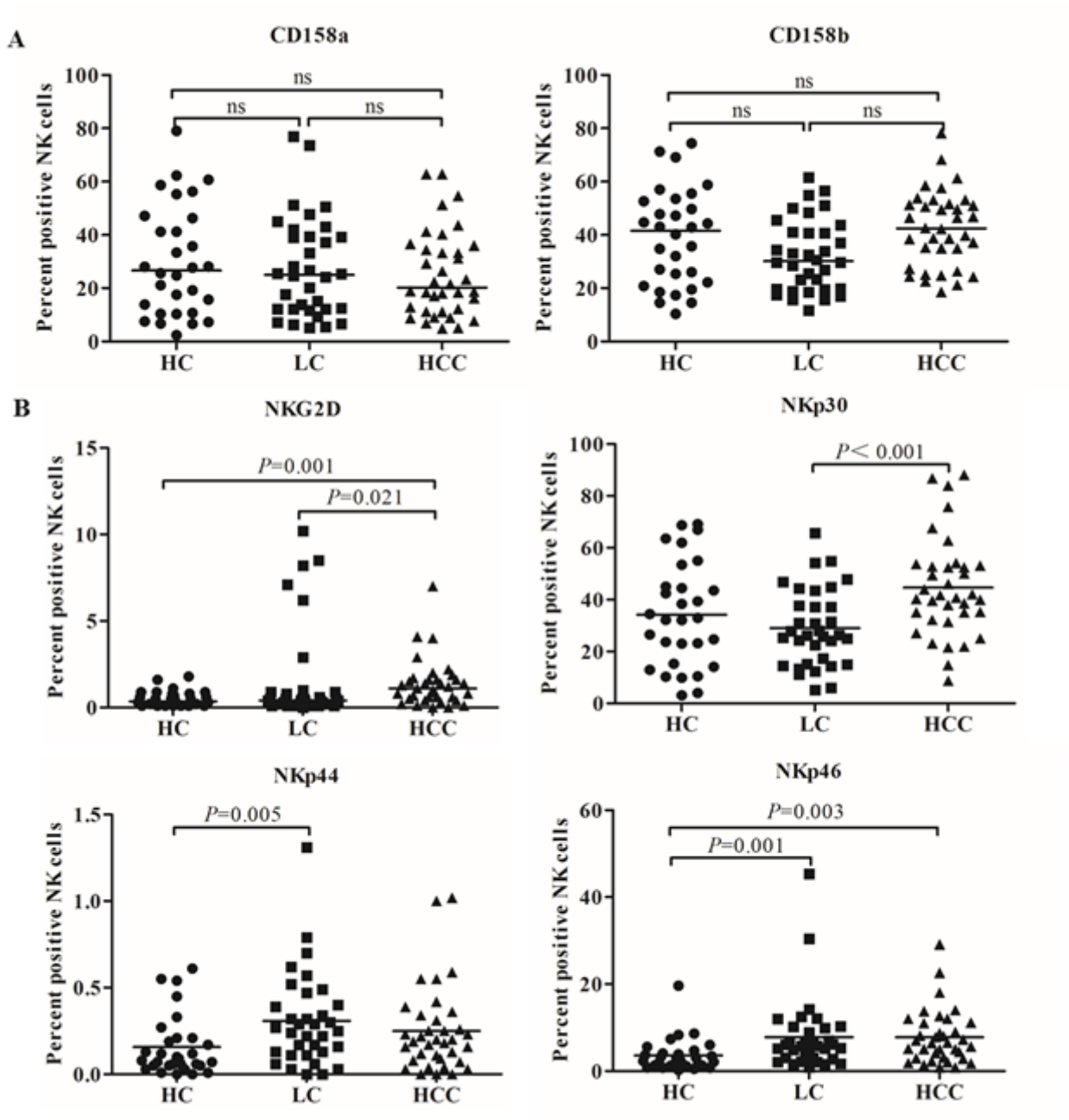

Figure 2

The proportion of activating receptors expression and inhibitors expression in NK cells. (A) The proportion of natural killer cells which expressed inhibitory receptors of CD158a/b in HC, LC and HCC subpopulation. Ns means there was no statistical significance. (B) The proportion of natural killer cells which expressed activating receptors of NKp30/p44/G2D/p46 in three groups.

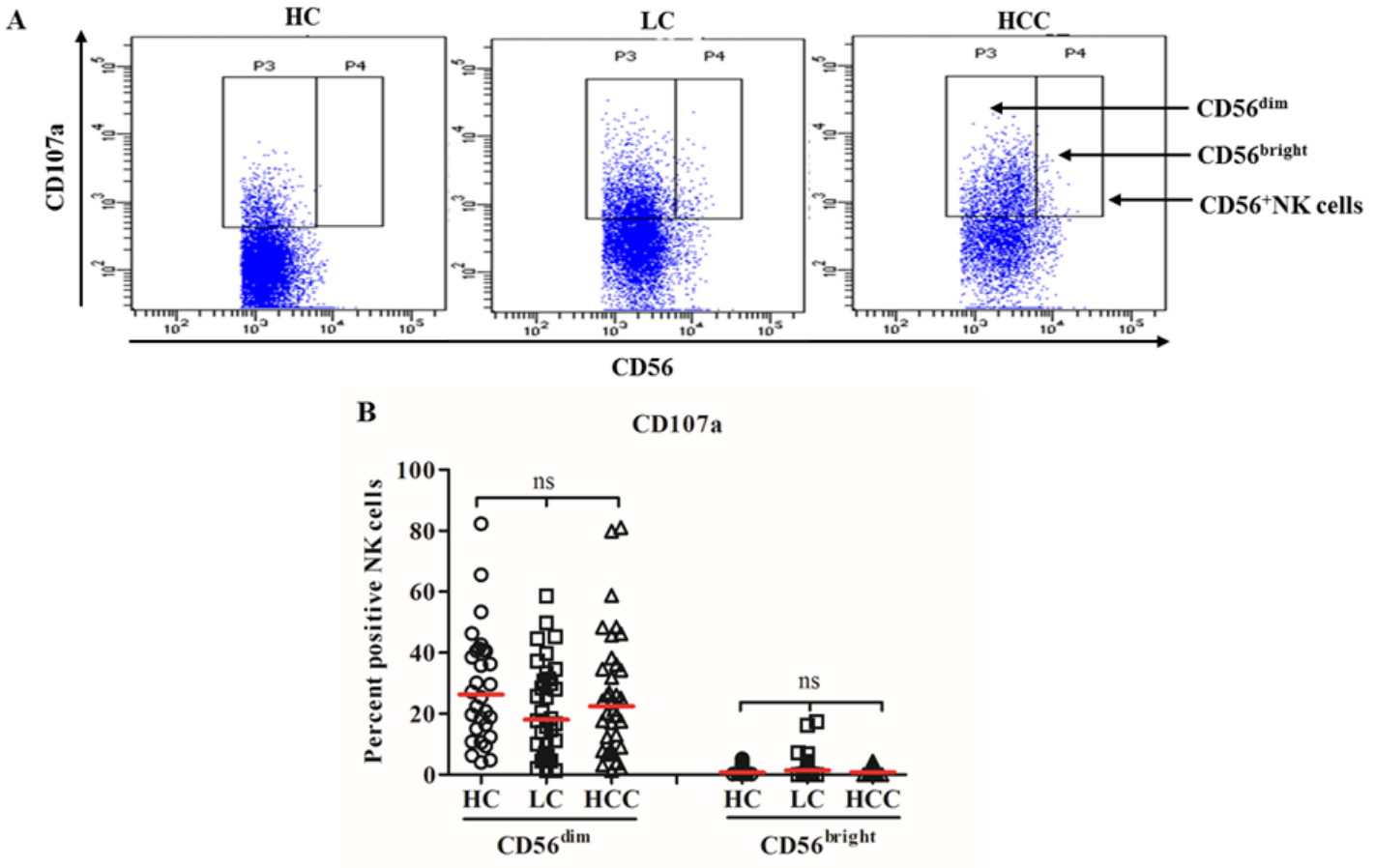

Figure 3

Degranulation of NK cells expressing CD107a. (A) Representative dot plots of NK cell expressing CD107a in three groups. (B) The percentage of NK cells expressing CD107a in three groups. Ns means there was no statistical significance.

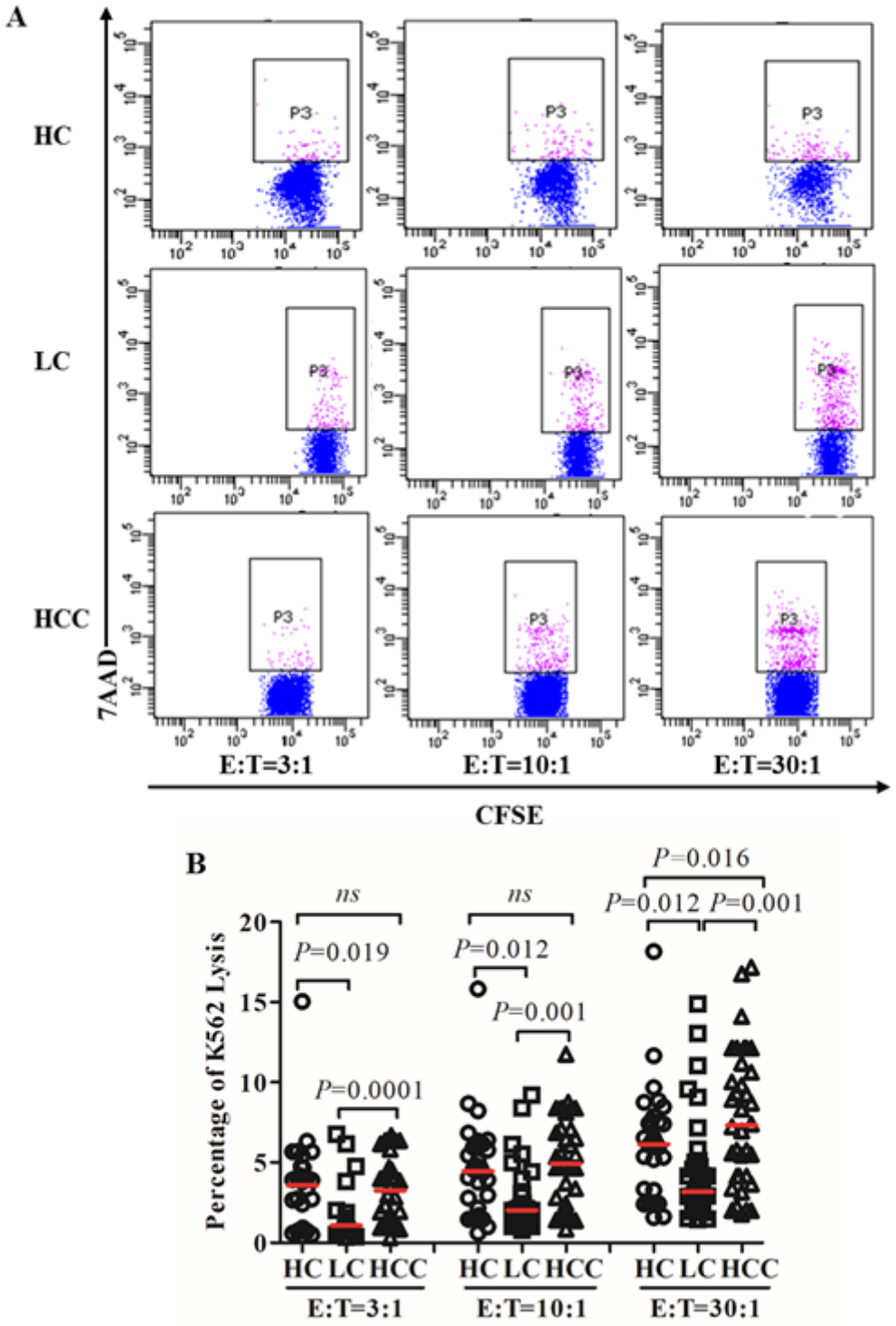

Figure 4

Cytolytic activity of NK cells among the three groups. (A) Dot plots of the K562 lysis proportion at the E:T ratio of 3:1, 10:1 and 30:1 within all the enrolled three subpopulations. (B) The proportion of K562 lysis proportion at the E:T ratio of 3:1, 10:1 and 30:1 in each subgroup, separately. Ns means there was no statistical significance.