Frequency and Implications of Natural Killer and Natural Killer T Cells in Hepatocellular Carcinoma

Article *in* Egyptian journal of Immunology · June 2018

CITATIONS
20

READS
318

6 authors, including:

Asmaa M Zahran
Assiut University
166 PUBLICATIONS **1,406** CITATIONS
SEE PROFILE

Amal Rayan
Assiut University
38 PUBLICATIONS **240** CITATIONS
SEE PROFILE

Nahla Elsherbiny
Assiut University
26 PUBLICATIONS **405** CITATIONS
SEE PROFILE

Helal F Hetta
University of Cincinnati Medical Center
312 PUBLICATIONS **5,919** CITATIONS
SEE PROFILE

Frequency and Implications of Natural Killer and Natural Killer T Cells in Hepatocellular Carcinoma

Asmaa M Zahran[1], Mona M. Abdel Meguid[2], Ahmed M. Ashmawy[3], Amal Rayan[4], Azza Elkady[5], Nahla M. Elsherbiny[6], Helal F. Hetta[6]

[1]Department of Clinical Pathology, South Egypt Cancer Institute, [2]Department of Clinical & Chemical Pathology, Faculty of Medicine, Al-Azhar University, [3]Department of Internal Medicine, Faculty of Medicine, Assiut University, [4]Department of Clinical Oncology, Faculty of Medicine, Assiut University, [5]Sohag General Hospital and [6]Department of Medical Microbiology & Immunology, Faculty of Medicine, Assiut University, Egypt.

A complex role of the immune system has been highlighted in the development and progression of hepatocellular carcinoma (HCC). Natural Killer cells (NK) and natural killer T cells (NKT) cells are among the innate immune lymphocytes that predominate in the liver and can prevent tumor growth and metastasis. The aim of the study was to measure the percentages of NK and NKT cells among a sample of Egyptian patients with HCC and to find the association between their frequencies and disease progression. The study included 2 groups; the HCC patient group (n=40) and the healthy control group (n=20). Blood samples were drawn from all subjects for complete blood picture, liver enzymes and alpha fetoprotein serum level measurement. Flow cytometric analysis was performed for CD3 and CD16/56 for determining the percentages of NK and NKT cells. The frequencies of NK cells and NKT cells were significantly decreased in HCC patients (6.58± 1.76 and 5.26 ± 1.13 respectively) as compared to healthy controls (9.01±1.62 and 6.88 ± 1.88 respectively) (*P*<0.001 and 0.0008 respectively) and in HCC patients with metastasis (6.01± 1.11 and 5.07± 1.10 respectively) than HCC patients without metastasis (7.75± 1.98 and 5.89± 0.88 respectively) (*P*= 0.004 and 0.03 respectively). We concluded that the reduced percentages of NK cells and NKT cells in HCC patients especially in those with metastasis point to their important roles in the occurrence and progression of HCC.

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide especially in developing countries, where hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are endemic [1]. In Egypt, HCC constitutes 70.48% of all hepatic tumors [2]. It has a very poor prognosis, and most patients are diagnosed at advanced stages leading to limited therapeutic options [3, 4].

A complex role of the immune system has been highlighted in the development and progression of HCC in many studies making immunotherapy a promising adjuvant therapy for such patients [5, 6]. It is now being recognized that the innate arm of the immune system may have a potentially important role in immune responses in liver diseases [7].

The distribution of lymphocytes in the liver differ from the peripheral blood and other organs [8-11]. The liver is characterized by a high proportion of the innate immune lymphocytes [including the natural killer (NK) and natural killer T (NKT) cells] compared to other organs pointing to their critical roles in the pathogenesis and immune homeostasis in the liver [12-14]. These cells are among the important cytolytic components in the liver and are able to release immunomodulatory cytokines, which activate other cells of the immune system preventing tumor growth and spread. Therefore, a decrease in the

count of these cells may facilitate tumor progression by unknown mechanisms [15-18].

Many studies pointed to the lower representation of the NK and NKT cells in the tumor environment which was also negatively associated with the prognosis of HCC patients [19]. On the other hand, few studies referred to their pattern in the peripheral blood of patients with HCC [12, 21]. The study aimed to evaluate NK and NKT cells among Egyptian patients with HCC and to find a possible association between their frequencies and disease progression.

Patients and Methods

Study subjects

This is a prospective case–controlled study that included 40 patients with newly diagnosed HCC in Assiut university hospitals, Faculty of Medicine, Assiut University and 20 age and sex matched healthy controls. The HCC group included 26 patients with metastasis and 14 patients without metastasis. All patients didn't receive any anti-tumor treatment. The study was approved by the local ethical committee of Faculty of Medicine, Assiut University. An informed written consent was taken from of all cases and controls. Diagnosis of HCC was achieved by triphasic CT abdomen or dynamic MRI demonstrating the criteria of HCC which are rapid arterial enhancement and late venous washout. The severity of cirrhosis was assessed by The Child-Pugh score. Exclusion criteria included HCC patients under treatment with immunosuppressives or chemotherapy, previously treated patients, other malignancies and chronic diseases.

Five ml peripheral blood samples were taken from all subjects and complete blood picture by the fully automated blood counters (Celltac E automated hematology analyzer, Tokyo, Japan), liver functions tests (alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by Cobas Integra 400 Chemistry Analyzer (Roche Diagnostics GmbH, Mannheim, Germany) and alpha fetoprotein serum level measurement using Access 2 tumor marker analyzer (Beckman Coulter, USA, S.N.: 510552) were done.

Flow cytometric detection of NK and NKT lymphocyte

Fifty µl of blood sample was stained with 5 µl of fluoroisothiocyanate (FITC)-conjugated CD3for identification of T lymphocytes and phycoerythrin (PE)-conjugated CD16/56 (Becton Dickinson (BD) Biosciences, San Jose, CA, USA) for detection of NK and NKT cells. Incubation was carried out for 15 minutes at room temperature in the dark, after which red blood cells lysis was done. Then the cells were washed once and were resuspended in phosphate buffer saline (PBS). Flow cytometric analysis was done by FACSCalibur flow cytometry with Cell Quest software (BD Biosciences, USA). Lymphocyte population was detected on the scatter histogram. Then, the percentages of $CD3^{-}CD16/56^{+}$ (NK) and $CD3^{+}CD16/56^{+}$ (NKT) were detected in the lymphocyte populations.

Statistical Analysis

Statistical analysis was performed with GraphPad Prism version 7.0 b software (Graph Pad Software Inc., San Diego, CA, USA). For qualitative data, the frequency and percentage was used, while for quantitative data, the mean± standard error (SEM) was used. Mann-Whitney analysis and Spearman's correlation were used to detect the statistical significant differences between groups and correlation analysis respectively. P value ≤ 0.05 was considered significant.

Results

The biochemical and clinical characteristics of the studied groups are summarized in table 1. The levels of total bilirubin, albumin, ALT, AST and AFP were significantly different in the HCC group as compared to the control group ($P<0.001$) as shown in table 1.

Table 1. Clinical and biochemical characterization of HCC patients and controls

	HCC group (n=40)	Controls (n=20)	*P*-value
Age (range)	50-70	53-65	NS
Sex (male/ female)	16/ 4	14/6	NS
Total bilirubin (mg/dl)	1.62±1.67	0.77±0.08	<0.001
Albumin (gm/dl)	3.04±0.7	4.08 ± 0.41	<0.001
ALT(mg/L)	136.82±17.12	29.70 ± 6.52	<0.001
AST (mg/L)	127.90±46.47	38.55 ± 11.06	<0.001
AFP (IU/L)	4383±763.40	5.23±1.80	<0.001
Child Pugh class	Class A (n=20) Class B (n=10) Class C (n=10)	na	na
Hepatic focal lesions	single < 5 cm (n=15) single >5 cm (n=10) multiple (n=15)	na	na
HCC &Metastasis			
With-metastasis	26/40 (65%)	na	na
Without metastasis	14/40 (35%)		

P>0.05 is not significant (NS), na = not applicable.

Frequency of CD3 T, NK and NKT cells among the studied groups

Lymphocyte population was detected on the scatter histogram. Then, the percentages of $CD3^{-}CD16/56^{+}$ (NK) and $CD3^{+}CD16/56^{+}$ (NKT) were detected in the lymphocyte populations (figure 1).

The frequencies of CD3 T, NK and NKT cells were significantly lower HCC patients compared to the control group (*P* values 0.04, ≤ 0.001, 0.0008) as shown in table 2.

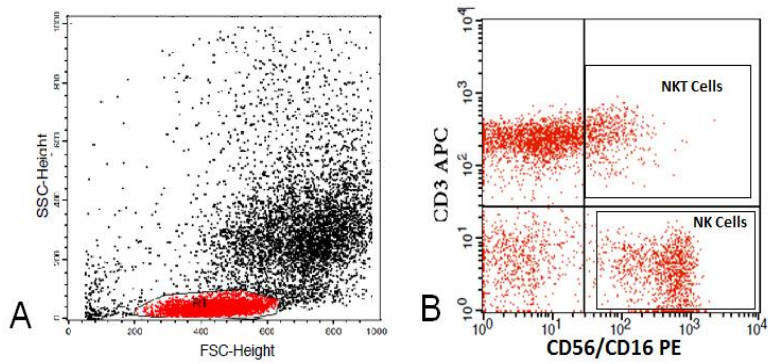

Figure 1. Flow cytometric analysis of NK and NKT lymphocytes

A: Forward and side scatter histogram to define the lymphocytes population (R1).

B: The expression of CD16/56 and CD3 in lymphocytes population

Table 2. The frequencies of CD3T, NK and NKT-cells among the studied groups

percentage	HCC patients (n=40)	Healthy controls (n=20)	*P- value
CD3 T cells	68.56±1.59	71.69±1.57	0.04
NK cells	6.58±1.76	9.01±1.62	<0.001
NK-T cells	5.26±1.13	6.88±1.88	0.0008

* *P*- value ≤0.05 is significant

Interestingly, the frequencies of NK cells and NKT cells were significantly lower in HCC patients with metastasis (6.01± 1.11 and 5.07± 1.10 respectively) compared to HCC patients without metastasis (7.75± 1.98 and 5.89± 0.88 respectively) (*P*= 0.004 and 0.03 respectively) as shown in figure 2.

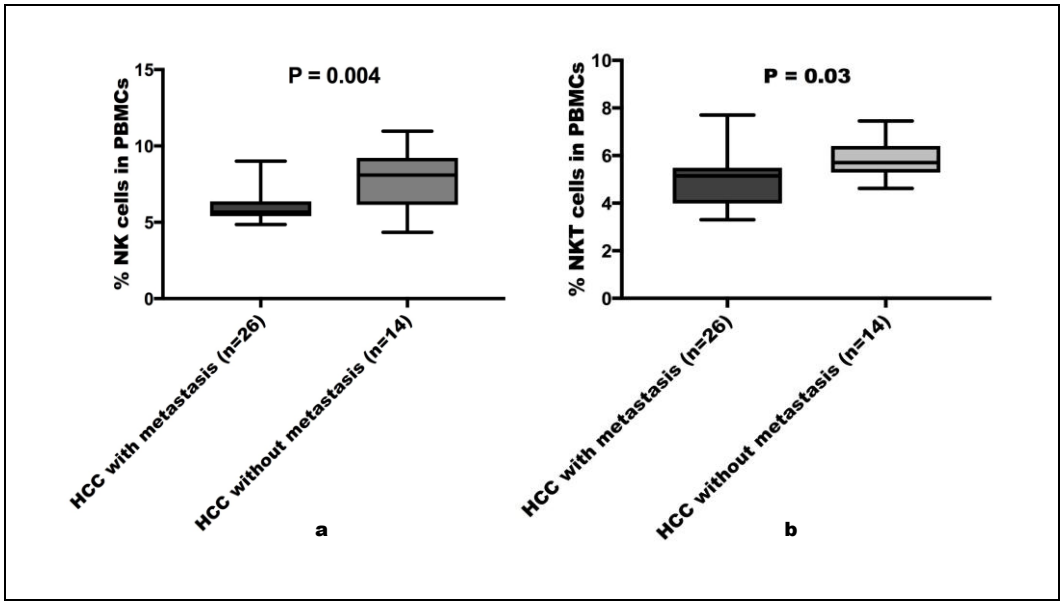

Figure 2. Frequencies of NK and NKT cells in HCC patients with and without metastasis.

Discussion

NK and NKT cells have important roles in defense against viral infections and tumors [21]. In the liver, a high proportion of NK (30-50%) and NKT cells (5-10%) have been recognized [12, 22]. Therefore, the decrease in the count of these cells by different mechanisms may facilitate the tumor progression. Many studies demonstrated a lower percentage of NK and NKT cells in the tumor infiltrating lymphocytes (TILs) which was negatively associated with the prognosis of HCC patients [19, 20].However, there has been a debate regarding their frequencies in the peripheral blood of HCC patients. So, we aimed to evaluate the percentages of NK and NKT cells among Egyptian patients with HCC and to find the association between their frequencies and disease progression.

In the present study, there was a significant decrease in the frequencies of NK and NKT cells in peripheral blood of HCC patients compared to controls (*P* values ≤ 0.001 and 0.0008).These findings are supported by previous studies [12, 22- 24].

In addition to the quantitative reduction in the NK cell, functional impairment was also reported previously leading to the loss of anti-tumor immune responses in HCC patients. The mechanisms responsible for impaired NK function remain unknown in HCC patients [22]. These cells are potent constitutive immune effectors, which can use the perforin-mediated secretory/necrotic mechanism in addition to the powerful TNF family ligand-mediated non-secretory apoptotic mechanism to destroy most solid tumor cell targets [25, 26]. In HCC patients, decrease in granule-releasing capacity of NK cells, alteration in the pattern of expression of NK cell-activating ligands, impairment of NK receptor-mediated signaling and increased Treg frequency might impair NK-mediated immune responses in these patients especially in advanced stages [27-29]. Also, phagocytosis of NK cells via hepatic stellate cells has been observed in patients with cirrhosis and HCC [30-31].

On the other hand, a recent study found that the proportions of NK and NKT cells were not altered in the peripheral blood of HCC as compared to healthy controls. The authors explained that by the relatively low proportion of NK and NKT cells in peripheral blood mononuclear cells of the healthy donors included in their study [12].

As for NK cells, the number of circulating NKT cells (type I)in addition to their functions were significantly lower in patients suffering from malignancies compared to controls [32-33]. The count of such cells was even lower in late-stage versus early-stage cancer patients [33].

In this study, we also found that the frequencies of the peripheral NK and NKT cells were significantly decreased in HCC patients with metastasis as compared to HCC patients without metastasis suggesting depletion by malignancy. This is in accordance with Guo *et al.,* who reported that the frequency of NK cells in TILs of HCC patients with metastasis was lower than those without metastasis [20].This was also supported by the findings of a previous experimental study [34]. Such reduction was attributed to the higher frequency of Treg cells in patients with metastasis than those without metastasis [20].

The anti-metastatic function of NK cells is mediated by the control of tumor metastasis through perforin and IFN-γ [35].Moreover it was previously reported that the frequencies of these innate immune lymphocytes in the HCC tissue are significantly associated with patients' survival making them good prognostic markers [20].

The role of NKT cells in cancer has been an important topic recently. Two subsets have been recognized (type I and type II) based on their TCR repertoire. Both subtypes recognize molecules presented by CD1d molecules but differ in the type of antigen to be recognized, for type I, it is glycosphingolipid α-galactosylceramide (α-GalCer), while for type II it is non-α-GalCer molecules [36].

In the peripheral blood, the circulating type I counts vary among healthy individuals ranging from <0.1% to more than 5% of the total T lymphocytes [37,38]. In the liver, type I NKT constitutes ~1% of total T lymphocytes [39]. Functionally, these cells are divided into 5 subsets depending on the cytokines they secrete (TH1- like, TH2-like, TH17-like, Treg-like, TFH-like). For Type II NKT cells which lack specific markers, only

2 subsets were determined (TH1- like and TH2-like).

Activated NKT cells can kill tumor cells directly in a CD1d-dependent manner unlike NK that use perforin/granzyme-mediated mechanisms mostly [40]. Activation depends on the balance between inhibitory and stimulatory signals as in NK cells leading to expansion and secretion of a number of cytokines [41, 42]. It was previously reported that the higher the numbers of such cells in the tumor, the better the clinical outcome [43]. The role of NKT cells in cancer is recently found to be more dynamic than previously thought. During the early stages of the tumor, TH1-like NKT cell subsets can initiate effective antitumor immune responses. However, as the tumor progresses, the NKT cells become over stimulated and anergic facilitating immune escape and losing their antitumor function [44].

We conclude that NK cells and NKT cells are found in lower frequencies in HCC patients compared to controls with even more significant reduction in HCC patients with metastasis compared to those without metastasis pointing to their significance in HCC development and progression.

References

1. El–Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterol; 2007, 132(7): 2557-2576.

2. Mokhtar N, Gouda I, Adel I. Cancer pathology registry 2003–2004 and time trend analysis. Department of pathology, NCI; 2007.

3. Weide B, Martens A, Zelba H, Stutz C, Derhovanessian E, Di Giacomo AM, Maio M, Sucker A, Schilling B, Schadendorf D, Büttner P, Garbe C, Pawelec G. l. Myeloid-derived suppressor cells predict survival of patients with advanced melanoma: comparison with regulatory T cells and NY-ESO-1- or melan-A–specific T cells. Clinical Cancer Research; 2014, 20(6): 1601-1609.

4. Hetta HF, Elkady A, Tohamy TA, Badary MS. Regulatory B Cells: Key Players in Hepatocellular Carcinoma Progression. Gastroenterology & Hepatology: Open Access; 2016, 5(2): 00136. DOI: 10.15406/ghoa.2016.05.00136.

5. Wirth TC. Spontaneous and therapeutic immune responses in hepatocellular carcinoma: implications for current and future immunotherapies. Expert review of gastroenterology & hepatology; 2014, 8(1): 101-110.

6. Breous E, Thimme R. Potential of immunotherapy for hepatocellular carcinoma. J Hepatol; 2011, 54(4): 830-834.

7. Doskali M, Tanaka Y, Ohira M, Ishiyama K, Tashiro H, Chayama K, Ohdan H. Possibility of adoptive immunotherapy with peripheral blood-derived CD3− CD56+ and CD3+ CD56+ cells for inducing antihepatocellular carcinoma and antihepatitis C virus activity. J Immunotherapy; 2011, 34(2): 129-138.

8. Hetta HF, Mehta MJ, Shata MTM. Gut immune response in the presence of hepatitis C virus infection. World J Immunol 2014; 4(2): 52-62.

9. Hetta HF, Mekky MA, Khalil NK, Mohamed WA, El-Feky MA, Ahmed SH, Daef EA, Medhat A, Nassar MI, Sherman KE, Shata MT. Extra-hepatic infection of hepatitis C virus in the colon tissue and its relationship with hepatitis C virus pathogenesis. J Med Microbiol; 2016, 65(8): 703-12.

10. Hetta, HF, Mehta MJ, Shata MT. Association of colonic regulatory T cells with hepatitis C virus pathogenesis and liver pathology. J Gastroenterol Hepatol; 2015, 30(10): 1543-51.

11. Mehta M, Hetta HF, Abdel-Hameed EA, Susan D. Rouster, MdMonir Hossain, Mohamed A. Mekky, Nasr K. Khalil, Wegdan A. Mohamed, Mohamed A. El-Feky, Shabaan H. Ahmed, Enas A. Daef, Mohamed A. El-Mokhtar, Sayed F. Abdelwahab, Ahmed Medhat, Kenneth E. Sherman, Mohamed Tarek M. Shata. Association between IL28B rs12979860 single nucleotide polymorphism and the frequency of colonic Treg in chronically HCV-infected patients. Arch Virol; 2016, 161(11): 3161-3169.

12. Li XF, Dai D, Song XY. A different representation of natural T cells and natural killer

cells between tumor-infiltrating and periphery lymphocytes in human hepatocellular carcinoma. Oncol Lett; 2017, 13(5): 3291-8.

13. Hoechst B, Voigtlaender T, Ormandy L, Gamrekelashvili J, Zhao F, Wedemeyer H, Lehner F, Manns MP, Greten TF, Korangy F. Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the NKp30 receptor. Hepatol; 2009, 50(3): 799-807.

14. Sun C, Sun HY, Xiao WH, Zhang C, Tian ZG. Natural killer cell dysfunction in hepatocellular carcinoma and NK cell-based immunotherapy. Acta Pharmacol Sin; 2015, 36(10): 1191-9.

15. Wu K, Kryczek I, Chen L, Zou W, Welling TH. Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. Cancer research; 2009, 69(20): 8067-8075.

16. Seki, S., Nakashima H, Nakashima M, Kinoshita M. Antitumor immunity produced by the liver Kupffer cells, NK Cells, NKT Cells, and $CD8^+$ $CD122^+$ T Cells. Clin & Developm Immunol; 2011, 2011.

17. Morishima C, Paschal DM, Wang CC, Yoshihara CS, Wood BL, Yeo AE, Emerson SS, Shuhart MC, Gretch DR. Decreased NK cell frequency in chronic hepatitis C does not affect ex vivo cytolytic killing. Hepatol; 2006, 43(3): 573-580.

18. Kawarabayashi N, Seki S, Hatsuse K, Ohkawa T, Koike Y, Aihara T, Habu Y, Nakagawa R, Ami K, Hiraide H, Mochizuki H. Decrease of CD56+T cells and natural killer cells in cirrhotic livers with hepatitis C may be involved in their susceptibility to hepatocellular carcinoma. Hepatol; 2000, 32(5): 962-969.

19. Gehring AJ, Ho ZZ, Tan AT, Aung MO, Lee KH, Tan KC, Lim SG, Bertoletti A. Profile of tumor antigen-specific CD8 T cells in patients with hepatitis B virus-related hepatocellular carcinoma. Gastroenterol; 2009, 137(2): 682-690.

20. Guo C-L, Yang HC, Yang XH, Cheng W, Dong TX, Zhu WJ, Xu Z, Zhao L. Associations between infiltrating lymphocyte subsets and hepatocellular carcinoma. Asian Pacific J Cancer Prev; 2012, 13(11): 5909-5913.

21. Gao B, Jeong WI, Tian Z. Liver: An organ with predominant innate immunity. Hepatology2008, 47(2): 729-36.

22. Cai L, Zhang Z, Zhou L, Wang H, Fu J, Zhang S, Shi M, Zhang H, Yang Y, Wu H, Tien P, Wang FS. Functional impairment in circulating and intrahepatic NK cells and relative mechanism in hepatocellular carcinoma patients. Clin Immunol; 2008, 129(3): 428-437.

23. Fu J, Xu D, Liu Z, Shi M, Zhao P, Fu B, Zhang Z, Yang H, Zhang H, Zhou C, Yao J, Jin L, Wang H, Yang Y, Fu YX, Wang FS. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. Gastroenterol 2007, 132(7): 2328-39.

24. Smyth MJ, Teng MW, Swann J, Kyparissoudis K, Godfrey DI, Hayakawa Y. CD4+CD25+ T regulatory cells suppress NK cell-mediated immunotherapy of cancer. J Immunol; 2006, 176(3): 1582-7.

25. Vujanovic NL, NagashimaS HR, Whiteside TL. Nonsecretory apoptotic killing by human NK cells. The Journal of Immunology; 1996, 157(3): 1117-1126.

26. Koga T, Harada H, Shi TS, Okada S, Suico MA, Shuto T, Kai H. Hyperthermia suppresses the cytotoxicity of NK cells via down-regulation of perforin/granzyme B expression. Biochemical and biophysical research communications; 2005, 337(4): 1319-1323.

27. Alter G, Malenfant JM, Altfeld M. CD107a as a functional marker for the identification of natural killer cell activity. J Immunological methods; 2004, 294(1-2): 15-22.

28. Terme M, Nathalie C, Behazine C, Averil M, Toshiaki O, Laurence Z. Regulatory T cells control dendritic cell/NK cell cross-talk in lymph nodes at the steady state by inhibiting CD4+ self-reactive T cells. J Immunol; 2008, 180(7): 4679-4686.

29. Ishiyama K, Ohdan H, Ohira M, Mitsuta H, Arihiro K, Asahara T. Difference in cytotoxicity against hepatocellular carcinoma between liver and periphery natural killer cells in humans. Hepatol; 2006, 43(2): 362-372.

30. Muhanna N, Doron S, Wald O, Horani A, Eid A, Pappo O, Friedman SL, Safadi R. Activation of hepatic stellate cells after phagocytosis of lymphocytes: A novel pathway of fibrogenesis. Hepatol; 2008, 48(3): 963-77.

31. Coulouarn C, Factor VM, Conner EA, Thorgeirsson SS. Genomic modeling of tumor onset and progression in a mouse model of aggressive

human liver cancer. Carcinogenesis; 2011, 32(10): 1434-40.

32. Crough T, Purdie DM, Okai M, Maksoud A, Nieda M, Nicol AJ. Modulation of human Vα24+ Vβ11+ NKT cells by age, malignancy and conventional anticancer therapies. Brit J cancer; 2004, 91(11): 1880.

33. Singh A, Shukla N, Das S. Altered invariant natural killer T cell subsets and its functions in patients with oral squamous cell carcinoma. Scand J Immunol. 2013, 78(5): 468-477.

34. Kobayashi N, Hiraoka N, Yamagami W, Ojima H, Kanai Y, Kosuge T, Nakajima A, Hirohashi S. FOXP3+ regulatory T cells affect the development and progression of hepatocarcinogenesis. Clin Cancer Resear; 2007, 13(3): 902-911.

35. Takeda K, Hayakawa Y, Smyth MJ, Kayagaki N, Yamaguchi N, Kakuta S, Iwakura Y, Yagita H, Okumura K.. Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. Nat Med; 2001, 7(1): 94-100.

36. Kumar V, Delovitch TL. Different subsets of natural killer T cells may vary in their roles in health and disease. Immunology; 2014, 142(3): 321-336.

37. Lynch L, O'Shea D, Winter DC, Geoghegan J, Doherty DG, O'FarrellyC. Invariant NKT cells and CD1d+ cells amass in human omentum and are depleted in patients with cancer and obesity. Euro J Immunol; 2009, 39(7): 1893-1901.

38. BandyopadhyayK, Marrero I, Kumar V. NKT cell subsets as key participants in liver physiology and pathology. Cell & Mol Immunol; 2016, 13(3): 337.

39. Wingender G, Kronenberg M. Role of NKT cells in the digestive system. IV. The role of canonical natural killer T cells in mucosal immunity and inflammation. Amer J Physiol-Gastrointestin and Liver Physiol; 2008, 294(1): G1-G8.

40. Wingender G, Krebs P, Beutler B, Kronenberg M. Antigen-specific cytotoxicity by invariant NKT cells in vivo is CD95/CD178-dependent and is correlated with antigenic potency. J Immunol. 2010, 185(5): 2721-2729.

41. Nagaleekar VK, Sabio G, Aktan I, Chant A, Howe IW, Thornton TM, Benoit PJ, Davis RJ, Rincon M, Boyson JE. Translational control of NKT cell cytokine production by p38 MAPK. J Immunol. 2011, 186(7): 4140-4146.

42. Long EO, Kim HS, Liu D, Peterson ME, Rajagopalan S. Controlling natural killer cell responses: integration of signals for activation and inhibition. Ann rev Immunol. 2013, 31: 227-258.

43. Tachibana T, Onodera H, Tsuruyama T, Mori A, Nagayama S, Hiai H, Imamura M. Increased intratumor Vα24-positive natural killer T cells: a prognostic factor for primary colorectal carcinomas. Clinical Cancer Research; 2005, 11(20): 7322-7327.

44. Krijgsman D, Hokland M, Kuppen PK. The role of natural killer T cells in cancer—A phenotypical and functional approach. Frontiers in immunology; 2018, 9: 367:1-21.