Impact of multiplicity of functional KIR-HLA compound genotypes on hepatocellular carcinoma

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Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; HCC, hepatocellular carcinoma; HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptors.

Natural killer (NK) cells are potential immune components against hepatocellular carcinoma (HCC) after curative hepatectomy. Patients at high risk of HCC recurrence can be identified by quantifying NK cell licensing. Therefore, therapeutic strategies that manipulate NK cell activity may possibly improve the prognosis of HCC patients.

NK cells are key components of the innate immune system that act against infectious and neoplastic cells. NK cell activation is dependent upon the inhibitory-activating receptor equilibrium. Killer cell immunoglobulin-like receptors (KIRs) are the most polymorphic among these receptors. They contribute to receptor–ligand interactions that determine NK cell responses by recognizing specific human leukocyte antigen (HLA) class I allotype ligands.

In 2006, Anfossi et al. reported an educational mechanism for human NK cells that involves self-specific inhibitory KIR and cognate HLA ligand interactions. This mechanism leads NK cells to acquire a higher resting response capacity,¹ consistent with the NK cell "licensing" mechanism in mice.² The polymorphic genes for KIRs and their cognate HLA ligands generate diverse immune responses. Ligand specificities for five inhibitory KIRs and the different strengths of licensing effects through different KIR-HLA ligand interactions have been defined. Thereafter, Yu et al. demonstrated that NK cells, which express multiple inhibitory KIRs for self-HLA ligands within variegated NK cell

repertoires, show a synergistic effect of licensing, i.e., the expression of progressively higher numbers of self-reactive inhibitory KIRs is correlated with increased effector capacity.³ This quantitative effect of NK cell licensing has been verified consistently in mice by analyzing the functional influence of the multiple interactions of self-major histocompatibility complex (MHC)-specific inhibitory receptors.⁴ Recently, Beziatz et al. also showed a linear effect of KIR gene copy number variation and HLA ligands on the overall functional responsiveness of the NK cell repertoire by analyzing KIR-HLA genotypes of healthy human volunteers.⁵ Despite the accumulating evidence suggesting a quantitative effect of NK cell licensing in basic studies, its clinical impact on neoplastic diseases has never been demonstrated. This year, we were the first to report the impact of multiplicity of functional KIR-HLA compound genotypes on HCC recurrence after curative hepatectomy.⁶ The presence of KIR2DL1-C2, KIR2DL2-C1, KIR3DL1-BW4, or KIR3DL2-A3/11, functional compound genotypes that intrinsically license NK cells, did not markedly affect

HCC recurrence. However, the cumulative risk of recurrence in patients with at least three compound genotypes (highly licensed patients) was significantly lower than that in patients with one or two comgenotypes (poorly licensed pound patients), suggesting that the effect of NK cell licensing on HCC recurrence is quantitative. This result matches the previously postulated theory that the number and type of host MHC class I alleles quantitatively tune the responsiveness of individual NK cell subsets expressing the corresponding KIR.⁵ Highly licensed patients may equip NK cells with more vigorous immune-surveillance activity to act as potent constitutive immune effectors against both intrahepatic metastasis and de novo carcinogenesis, as compared to poorly licensed patients (Fig. 1).

We have previously shown that the distribution of NK cells is anatomically biased, e.g., NK cells are quite abundant in human livers.⁷ Functionally, liver NK cells display a significantly higher cytotoxic activity against neoplastic cells, including against HCC, through a tumor necrosis factor-related apoptosisinducing ligand-mediated mechanism,

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Figure 1. NK cell licensing depends on the number of functional KIR–HLA compound genotypes. The quantitative effects of NK cell licensing influence the overall functionality of NK cell-mediated tumor surveillance. Increasing the number of functional KIR–HLA compound genotypes stochastically induces the generation of NK cell repertoires, which progressively express self-reactive inhibitory KIRs. The difference in NK cell repertoires potentially generates different NK cell surveillance functions, e.g., abundant recruitment of highly licensed NK cells prevents carcinogenesis through attacks on circulating metastatic cells in sinusoid or *de novo* neoplastic cells.

than peripherally circulating NK cells. Based on this fact and the evidence of a quantitative effect of NK cell licensing, we wondered whether the multiplicity of functional KIR–HLA compound genotypes might exclusively influence intrahepatic carcinogenesis or metastasis, regardless of their origin. It would be of great interest to define the impact of the quantitative effect of NK cell licensing on intrahepatic cholangiocarcinoma, liver metastasis of colorectal cancer, or other malignancies.

We demonstrated the predictive value of the multiplicity of functional KIR– HLA compound genotypes for HCC recurrence after curative hepatectomy. One potential strategy to compensate for the genetic susceptibility to HCC recurrence would be an immune therapy that manipulates the NK cell activity. To this end, the clinical efficacy of adoptive immunotherapy with interleukin-2 and anti-CD3 monoclonal antibody-activated autologous peripheral lymphocytes, such as NK cells, has been evaluated; prolonged relapse-free survival in HCC patients following resection of the primary tumor was analyzed. 8 However, the details of the mechanisms underlying such effects remain unclear. As liver NK cells display more vigorous cytotoxicity against HCC than peripheral NK cells, we recently proposed a novel type of adjuvant

immunotherapy for preventing HCC recurrence in liver transplant recipients. In this immunotherapy, transplant recipients are intravenously injected with lymphokine-activated killer cells, including activated NK cells derived from liver allografts.⁷ A clinical phase I trial revealed the feasibility and safety of this immune therapy.⁹ To define the long-term benefits of this approach in terms of the control of HCC recurrence after liver transplantation, a phase II trial, which will investigate the influence of the KIR–HLA genotypes in donors and recipients of liver transplants, is currently under consideration.

Antibody targeting agents for cancer treatment use both compliment-mediated

cytotoxicity and antibody-dependent cellmediated cytotoxicity (ADCC) to lyse antibody-coated cells. Since NK cells contribute to ADCC, therapies combining NK cells and antibody targeting have the added advantage of local activation of NK cells at the tumor site via CD16

References

- Anfossi N, Andre P, Guia S, Falk CS, Roetynck S, Stewart CA, Breso V, Frassati C, Reviron D, Middleton D et al. Human NK cell education by inhibitory receptors for MHC class I. Immunity 2006; 25:331-42; PMID:16901727; http://dx.doi.org/10.1016/j. immuni.2006.06.013
- Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L, French AR, Sunwoo JB, Lemieux S, Hansen TH et al. Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature 2005; 436:709-13; PMID:16079848; http://dx.doi.org/10.1038/nature03847
- Yu J, Heller G, Chewning J, Kim S, Yokoyama WM, and Hsu KC. Hierarchy of the human natural killer cell response is determined by class and quantity of inhibitory receptors for self-HLA-B and HLA-C ligands. J Immunol 2007; 179:5977-89; PMID: 17947671; http://dx.doi.org/10.4049/jimmunol. 179.9.5977
- Brodin P, Lakshmikanth T, Johansson S, Karre K, and Hoglund P. The strength of inhibitory input during education quantitatively tunes the functional

activation. A previous report demonstrated that unlicensed NK cells formed the predominant subset of NK cells, which have potent ADCC due to their lack of inhibitory receptors for self.¹⁰ Hence, the impact of NK cell licensing on the therapeutic outcome of antibody

responsiveness of individual natural killer cells. Blood 2009; 113:2434-41; PMID:18974374; http://dx.doi. org/10.1182/blood-2008-05-156836

- Beziat V, Traherne JA, Liu LL, Jayaraman J, Enqvist M, Larsson S, Trowsdale J, and Malmberg KJ. Influence of KIR gene copy number on natural killer cell education. Blood 2013; 121:4703-7; PMID: 23637128; http://dx.doi.org/10.1182/blood-2012-10-461442
- Tanimine N, Tanaka Y, Kobayashi T, Tashiro H, Miki D, Imamura M, Aikata H, Tanaka J, Chayama K, and Ohdan H. Quantitative effect of natural killer cell licensing on hepatocellular carcinoma recurrence after curative hepatectomy. Cancer Immunol Res 2014; 2(12):1142-7; PMID:25135909; http://dx.doi.org/ 10.1158/2326-6066.CIR-14-0091
- Ishiyama K, Ohdan H, Ohira M, Mitsuta H, Arihiro K, and Asahara T. Difference in cytotoxicity against hepatocellular carcinoma between liver and periphery natural killer cells in humans. Hepatology 2006; 43:362-72; PMID:16440347; http://dx.doi.org/ 10.1002/hep.21035

targeting agents may need to be elucidated through further studies.

Disclosure of Potential Conflicts of Interest

The authors have declared no conflicts of interest.

- Takayama T, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J, Shimada K, Sakamoto M, Hirohashi S, Ohashi Y et al. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. Lancet 2000; 356: 802-7; PMID:11022927; http://dx.doi.org/10.1016/ S0140-6736(00)02654-4
- Ohira M, Ishiyama K, Tanaka Y, Doskali M, Igarashi Y, Tashiro H, Hiraga N, Imamura M, Sakamoto N, Asahara T et al. Adoptive immunotherapy with liver allograft-derived lymphocytes induces anti-HCV activity after liver transplantation in humans and humanized mice. J Clin Invest 2009; 119:3226-35; PMID:19805910; http://dx.doi.org/ 10.1172/JCI38374
- Tarek N, Le Luduec JB, Gallagher MM, Zheng J, Venstrom JM, Chamberlain E, Modak S, Heller G, Dupont B, Cheung NK et al. Unlicensed NK cells target neuroblastoma following anti-GD2 antibody treatment. J Clin Invest 2012; 122:3260-70; PMID: 22863621; http://dx.doi.org/10.1172/JCI62749