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Adoptive transfer of natural killer cells in combination with chemotherapy improves outcomes of patients with locally advanced colon carcinoma

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Abstract

Background. Despite the availability of multiple treatment strategies, patients with advanced colon carcinoma (CC) have poor prognoses. The aim of this study was to evaluate the efficacy and safety of natural killer (NK) cell therapy in combination with chemotherapy in patients with locally advanced CC. *Methods.* We assessed the cytotoxicity of NK cells to CC cells (CCs) and CC stem cells (CSCs) pre-treated with 5-fluorouracil or oxaliplatin *in vitro*. Then, an open-label cohort study was conducted with locally advanced CC patients who had received radical resection. Patients received either NK cell therapy combined with chemotherapy (NK cell group, 27 patients) or pure chemotherapy (control group, 33 patients). Progression-free survival (PFS), overall survival (OS) and adverse effects were investigated. *Results.* Chemotherapy sensitized CCs and CSCs to NK cell cytotoxicity through regulation of NK cell–activating/inhibitory receptor ligands. Poorly differentiated CCs were more susceptible to NK cells than well-differentiated ones. In the cohort study, the 5-year PFS and OS rates in the NK cell group were significantly higher than those in the control group (51.1% versus 35%, $P = 0.044$; 72.5% versus 51.6%, $P = 0.037$, respectively). Among patients with poorly differentiated carcinomas and low expression of human leukocyte antigen (HLA)-1, the median PFS in the NK cell group versus the control group was 23.5 versus 12.1 months ($P = 0.0475$) and 33.1 versus 18.5 months ($P = 0.045$), respectively. No significant adverse reactions were reported. *Conclusion.* NK cell therapy in combination with chemotherapy in locally advanced CC prevented recurrence and prolonged survival with acceptable adverse effects, especially for poorly differentiated carcinomas.

Key Words: *chemotherapy, colon cancer, natural killer cell therapy*

Introduction

Colon cancer (CC) is one of the major causes of cancer-related deaths worldwide, with over 500,000 deaths occurring each year, especially among patients with advanced CC [1,2]. CC is generally difficult to detect and diagnose until symptoms become apparent, at which point the disease may have reached an advanced stage. Among patients with advanced CC, metastatic diseases (stage IV) are frequently incurable, so we focus on these locally advanced CC (stage III) patients with more therapeutic significance here [3]. Despite the application of surgical excision combined with various adjuvant therapies, such as radiotherapy, chemotherapy or targeted therapy, the prognoses of these patients are still disappointing [4,5]. This is primarily due to the resistance of residual CC cells (CCs) to current anti-cancer therapies, thereinto, a relatively higher proportion of colon cancer stem cells (CSCs) in advanced CC compared with early-stage CC may take the most responsibility for the poor prognosis of advanced CC [6,7]. Therefore, novel therapies against CSCs are urgently needed to improve the outcome of locally advanced CC.

Cancer immunotherapies are a potential approach for eliminating CSCs owing to the reported role of immune effector cells, such as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, in the prevention of tumor formation [8]. Although CTL-based vaccines showed promise in pre-clinical studies [9], the clinical outcomes of these immunotherapies have been disappointing, partly because of the complexity of immune escape mechanisms of tumor cells, including the down-regulation of major histocompatibility complex (MHC) molecules and target antigens on tumor cells [10]. Therefore, non-MHC–restricted innate immunotherapy could be a novel strategy to treat patients with advanced CC. Therefore,

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non-MHC–restricted NK cells, classically considered effector cells of cellular immunotherapy, have garnered interest for their properties of adoptive immunity to induce long-lasting specific responses [11,12].

NK cell–mediated targeted cell lysis and apoptosis are a cell contact–dependent process through which NK cells activate receptors and target cell ligand interactions to perform direct potent cytotoxic activity via perforin/granzyme, Fas/FasL and tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL)/TRAIL receptor (TRAIL-R) pathways [13–16]. This activity is termed the "cancer immunosurveillance of NK cells" [17]. At the same time, activity of NK cells can be inhibited by a surplus of inhibitory ligands on tumor cells, such as MHC class 1, allowing tumor cells to escape from NK cells immunosurveillance [18]. Whether NK cells play a positive role in cancer immunosurveillance depends on the net input of activating and inhibitory signals perceived by target cells through NK cell surface receptors. Thus, a proportion of NK cell–activating and inhibitory receptor ligands on tumor cells may play a decisive role, deciding whether target tumor cells are susceptible to NK cells.

Fortunately, postoperative chemotherapy, a common adjuvant therapy for locally advanced CC, plays a role in killing residual tumor cells while enhancing the efficacy of immunotherapy by reducing postoperative immunosuppression [19,20]. Furthermore, some chemotherapeutics, such as 5-fluorouracil (5-Fu), have been reported to regulate expression of NK cell receptor ligands on tumor cells to sensitize them to lysis of NK cells [21]. Therefore, we hypothesized that chemotherapy in combination with an infusion of NK cells following surgical excision would significantly improve the prognoses of patients with locally advanced CC compared with that of postoperative pure chemotherapy.

Recently, some studies have suggested that a higher quantity of intratumoral NK cells might be associated with better prognosis of colorectal cancer [22,23]; however, there has not yet been a systematic and comprehensive study to confirm that NK cell immunotherapy results in favorable responses in these patients.

In this study, we first demonstrated that both CCs and their CSCs can be recognized and killed by NK cells and that their susceptibility to NK cell–mediated cytolysis could be significantly enhanced after pre-treatment with chemotherapy through *in vitro* experiments. We then conducted a pilot prospective cohort study to verify the safety and efficacy of NK cell therapy combined with chemotherapy in patients with locally advanced CC, with the objective of improving remission and prolonging survival rates in patients who had received surgical excision.

Material and methods

Patients and clinical assessment

This study was an open-label prospective cohort study, and the study protocol was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the institutional ethics committee of the First Hospital of Jilin University (Number 2009-012).

All patients with CC who met the following criteria between January 2010 and November 2011 were included: (i) ≥ 18 years of age with primary locally advanced CC (stage IIIa–c); (ii) an Eastern Cooperative Oncology Group (ECOG) performance status ≤2; and (iii) had received radical resection. Exclusion criteria included the following: (i) other immunotherapies; (ii) clinically serious infections; (iii) a history of organ transplantation; and (iv) pregnancy or breastfeeding. After radical resection therapy, patients directly chose to either receive chemotherapy combined with NK cell therapy (at least two courses) as part of the study group, or pure chemotherapy as part of the control group. The decision whether to undergo NK cell therapy or to be entered into the control group was made by the patient. All enrolled patients received standard first-line chemotherapy within 3–4 weeks after enrollment, including 5-Fu and oxaliplatin (L-OHP), according to the National Comprehensive Cancer Network (NCCN) guidelines. Informed consent was obtained from all patients prior to enrollment. Furthermore, the CC tissues of enrolled patients were obtained from the surgical resection for further detection of NK cell receptor ligands. If recurrence or progression occurred in patients in either the NK cell or control group, the patients chose to be given a second- or third-line chemotherapy regimen, NK cell therapy or best support therapy (BST), depending on general health status and/or preference (shown in Supplementary Table S1).

The primary objective of clinical assessment was to observe progression-free survival (PFS) and overall survival (OS), and the disease progress was assessed according to Response Evaluation Criteria In Solid Tumors guidelines [24]. PFS was defined as the period between the enrollment of patients and the onset of progressive disease (PD) or death from any cause. OS was defined as the period from the day on which patients were enrolled to death from any cause. The secondary objective was to observe side effects in the two groups.

Cell culture and reagents

The human CC cell lines HCT-116 and HRT-18 were obtained from the American Type Culture Collection (ATCC) and were cultured in complete Dulbecco's Modified Eagle's Medium (DMEM; HyClone, GE