NK Cells Adjuvant Therapy Reveals Obvious Survival Benefits in a Gastric Mixed Signet Ring Cell Carcinoma Patient: A Case Report

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Case report

Keywords: natural killer cells adjuvant therapy, gastric signet ring cell carcinoma, distal gastrectomy, chemotherapy, tumor markers

Posted Date: December 2nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-117725/v1>

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Abstract

Background: Advanced signet ring cell (SRC) carcinoma has a worse prognosis. Therefore, early diagnosis and prevention is particularly important; SRC tumours have lower R0 resection rate and are thought to be less chemosensitive than non-SRCC. Consequently, a novel postoperative adjuvant treatment is urgently needed to improve clinical outcomes.

Case presentation: A 41-year-old female with paroxysmal vomiting for more than one year come to hospital for gastroscopy examination in 2015. The gastroscopy revealed signet ring cell carcinoma of gastric antrum and poorly differentiated adenocarcinoma in some areas. The patient was treated with radical gastrectomy for gastric cancer and oxaliplatin-based regimen for 6 cycles after surgery. Natural killer (NK) cells immunotherapy was started after November 2016, three times a year, up to now, no suspicious lesions were evident, and the levels of serum tumor markers were normal.

Conclusions: Intravenous injections of NK cells combination with surgical treatment and chemotherapy showed therapeutic effects in this patient with possible relapse.

Background

Gastric cancer (GC) is one of the most common malignancies worldwide, particularly in East Asia, East Europe, and South America [1]. Surgery is still the main treatment for operable GC. Postoperative chemotherapy is a recommended component of resectable GC therapy and has been shown to improve patient outcome in several studies. However, 30–50% of patients relapse within 5 years after surgery and adjuvant chemotherapy [2, 3]. Signet-ring cell (SRC) carcinoma, an unfavorable subtype of gastric cancer is a unique name for its particular histological features and clinical behavior. If treated early, signet ring cell carcinoma has a better prognosis than other subtypes; however, advanced signet ring cell carcinoma has a prognosis that is even worse than undifferentiated adenocarcinoma [4].

Early diagnosis of signet ring cell carcinoma is therefore critical to guide optimal treatment, but the typical presentation during conventional endoscopy is a pale, flat lesion, especially difficult to realize the early detection, so there is limitation to the early diagnosis, judgment of reoccurrence and evaluation of efficacy for tumors. The detection of tumor markers could reflect the occurrence and development of tumors timely and the detection process is convenient and rapid, with high sensitivity. Therefore, they have been the important clinical means of auxiliary examination in the clinical diagnosis and prognosis evaluation for tumors [5, 6]. Among the available tumor markers, carcino-embryonic antigen (CEA), carbohydrate antigen (CA) 19 – 9, and CA72-4 are widely used in the follow-up of patients with gastrointestinal malignancies. These markers have been demonstrated to be useful in the diagnosis, treatment and prognosis of GC [7, 8].

The potential of natural killer (NK) cells in comparison with other immune cells to induce cytotoxicity in the absence of prior sensitization caused them to be an attractive candidate for immunotherapy. Given the difficulties of sourcing abundant numbers of cytotoxic NK cells from peripheral blood, additional

strategies have been investigated to provide readily available banks of NK cells for patients. Umbilical cord blood (UCB) is an amazing source of NK cells for immunotherapy. The merits of UCB derived NK cells including availability of UCB units, high percentage of NK cell progenitors, collecting different source of UCB together, lower risk of graft versus host disease and less stringent requirements for HLA matching making UCB an off the shelf source for NK cells immunotherapy [9].

In view of this, we developed ex vivo expansion techniques that can induce cord blood mononuclear cells (MNC) to directly differentiate into high cytotoxic NK cells using a cocktail of cytokines and IL-2. The culture method of high cytotoxic NK cells will be introduced in another article. Here, we report a case of gastric mixed signet ring cell carcinoma, who received radical gastrectomy for gastric cancer and oxaliplatin-based chemotherapy before using our NK cells immunotherapy. In this study, we focuses on the survival benefits of NK cells immunotherapy and clinical utility of tumor markers CEA, alpha fetoprotein (AFP), CA15-3, CA12-5, CA19-9, CA72-4, human chorionic gonadotropin (HCG-BET), neuron-specific enolase (NSE) and potential blood biomarkers of T-cell subsets in monitoring response and predicting the prognosis of the patient.

Case Presentation

We report the case of a 41-year-old female. The patient presented paroxysmal vomiting for more than one year. She underwent gastroscopy evaluation and was diagnosed with gastric signet-ring cell carcinoma at Tangshan people's Hospital. Six days later, she was admitted to the Chinese PLA General Hospital and completed the preoperative examination. On December 30, 2015, she underwent radical (R0) gastrectomy under general anesthesia. The diagnosis of postoperative pathology report was as follows: ulcerative poorly differentiated adenocarcinoma in the lesser curvature of gastric antrum, most of them were signet ring cell carcinoma, only a small portion were mucinous adenocarcinoma, the tumor size was 7*6*1.8 cm, invading the full thickness of gastric wall, and the tumor thrombus can be seen in the vascular, no cancer tissue was found in the surgical margin of the upper and lower, but partial lymph node metastasis in the greater curvature of stomach. Immunohistochemically, the tumor showed positivity for cytokeratin, and the Ki-67 labeling index was 5%. Staining for human epidermal growth factor receptor-2 (HER-2) and human epidermal growth factor receptor-1 (HER-1) was negative. On the basis of the above findings, the tumor was diagnosed as gastric cancer with stage TC (T4a, N3a, M0). The patient was treated with oxaliplatin and tegafur combined chemotherapy for six cycles after the operation. The last chemotherapy time was on May 18, 2016. Serum samples for CEA, AFP, CA15-3, CA12-5, CA19-9, CA72-4, HCG-BET and NSE levels were measured during chemotherapy, and only the levels of CEA and CA72-4 were above the cut-off levels (Table 1). With the progression of chemotherapy, the level of CA72-4 decreased significantly (Table 1). However, the level of CA72-4 rapidly increased during the two follow-up after chemotherapy (Table 1). For fear of recurrence and metastasis after chemotherapy, the patient began to receive NK cells immunotherapy on November, 2016, using ex vivo-generated NK cells from UCB, at a dose of 2×10^9 CD56⁺/CD3⁻ cells, intravenously, 3 times a year, up to now. In the follow-up, the level of CA72-4 decreased rapidly, as of the latest follow-up, the level of CA72-4 had dropped to normal (Table 1) and she was

observed to be in a very good condition, without evidence of disease progression (V0, diagnosis; V1, radical gastrectomy and chemotherapy; V2, tumor markers detection and CT; V3-V8, NK cells therapy, tumor markers detection and CT, Figure. 1).

Table 1

Serum levels of CEA, AFP, CA15-3, CA12-5, CA19-9, CA72-4, HCG-BET and NSE at different time points.

Time	CEA ng/ml	AFP ng/ml	CA15-3 U/mL	CA12-5 U/mL	CA19-9 U/mL	CA72-4 U/mL	HCG-BET mIU/mL	NSE ug/L
2016-03-31	3.06	1.57	11.74	14.32	24.10	35.26↑	0.10	10.52
2016-05-17	4.22↑	1.59	10.70	12.99	27.49	18.24↑	0.10	12.12
2016-08-11	4.34↑	1.17	8.97	16.40	16.02	12.37↑	0.10	10.39
2016-09-20	2.62	-	-	-	-	46.67↑	-	-
2016-11-22	3.17	-	-	-	-	86.34↑	-	-
2016-11-30	2.10	1.67	7.64	20.60	14.87	16.68↑	0.10	8.27
2016-12-23	0.676	1.73	9.06	27.13	15.14	11.31↑	0.10	13.31
2017-02-10	-	-	-	-	-	12.82↑	-	-
2017-10-18	-	-	-	-	-	7.06↑	-	-
2018-11-06	2.84	1.48	8/39	13.12	17.42	6.68	0.889	9.79
2019-08-08	0.882	1.66	6.76	11.10	9.02	9.23↑	0/901	8.57
2020-09-08	0.91	1.10	6.89	9.68	8.73	3.82	1.06	8.64
The normal reference values were as follows: CEA $\leq$ 3.4 ng/ml; AFP $\leq$ 7 ng/ml; CA15-3 $\leq$ 25 U/mL; CA12-5 $\leq$ 35 U/mL; CA19-9 $\leq$ 39 U/ml; CA72-4 $\leq$ 6.9 U/ml; HCG-BET $\leq$ 3 mIU/mL; NSE $\leq$ 15.2 ug/L.								

Discussion And Conclusions

CA72-4 was first described by Colcher et al in 1981[10]. It is a glycoprotein with a molecular mass > 1000 kDa and is a tumor marker for numerous cancers, including breast, ovarian, colorectal and pancreatic cancer, and it has good specificity for GC [11, 12]. In the literature, CA72-4 was associated with advanced tumor stage, lymph node metastasis, and distant metastasis [13–19]. However, there are few studies on predictive screening or early detection particularly for CA72-4. The analysis showed that CA72-4 was the preferable single test, with a sensitivity value (93.83%) that was higher than that of CEA (72.20%) and much higher than that of CA19-9 (22.30%) [20]. In this study, CA72-4 appeared to be also the most sensitive and specific marker in the gastric cancer patient. At the beginning of treatment, the level of CA72-4 decreased from 35.26 U/mL to 18.24 U/mL, which can be seen as a response to treatment. However, the level of CA72-4 increased from 12.37 U/mL to 86.34 U/mL during the three

follow-up after chemotherapy. Because of the rapid increase of CA72-4 levels, we are worried about the risk of recurrence and metastasis despite the abdominal CT scan showed no abnormality.

On November 2016, the patient began to receive NK cells immunotherapy. In our study, we improved the preparation method of NK cells. Our technique resulted in a high yield of at least 1.0×10^{10} NK cells. These NK cells showed evident cytotoxicity against gastric cancer cell lines in vitro. One week after the NK cells infusion, the level of CA72-4 dropped to 16.68 U/mL (Table 1). For the next three years, the patient continued to receive NK cells immunotherapy. In the lately follow-up, the level of CA72-4 had dropped to the normal and the abdominal CT scan also found no abnormality.

Currently, surgical resection is still the primary treatment for many solid malignancies, however, scattered tumor cells that remain after resection of the primary tumor, may be the main trigger factor for disease recurrence [21]. Furthermore, surgical procedures may induce the release of immunosuppressing factors that render host immune surveillance ineffective, ultimately leading to the increased metastatic disease or recurrence following surgery [22]. Natural killer (NK) cells are cytotoxic lymphocytes that constitute a major component of the innate immune system. NK cell dysfunction following surgery has been documented in both human patients [23–25] and animal models [26, 27]. Rate of local recurrence following surgical tumor resection of colorectal cancer correlated with lower NK cell levels [28]. Correlations between reduced NK cytotoxicity and incidence of metastasis have been established in head and neck as well as pharyngeal cancer [29–31]. These examples highlight the potential for NK cell immunotherapies to improve patient outcomes [32].

Traditional postoperative adjuvant radio/chemotherapy may eliminate residual lesions and reduce tumor recurrence to some extent, but, pure adjuvant radio/chemotherapy primarily kills actively proliferating tumor cells rather than relatively indolent cancer stem cells, which are mainly responsible for recurrence [33, 34]. In addition, SRCC is thought to be less chemosensitive than non-SRCC. Therefore, a novel postoperative adjuvant treatment is urgently needed to improve clinical outcomes for these patients.

Pay an attention to the immune state of patients through detection of the level of lymphocyte subsets including percentage and number timely and accurately, it will help us to evaluate conditions of prognosis and adjust the treatment program for patients. Here we designed to investigate the percentages of CD3 + T, CD3 + CD4 + T, CD3 + CD8 + T, B and NK cells in peripheral blood of the patient using a single-platform flow cytometry-based method, and to analyze the immune function of the patient when the tumor marker CA72-4 increased to the level of 86.34 U/mL. As a result, a lower percentage of CD3 + T and NK cells were observed (Fig. 2), it states that the patient' immune function is impaired. However, there is untapped potential in the use of immunotherapies to reverse or prevent surgical stress-induced NK cell dysfunction. As for the critical role in the anti-tumor immune response of NK cells, we adopt the way of infusion the expansion of NK cells in vitro to repair the damaged immune function and prevent the recurrence and metastasis.

In conclusion, this is the first demonstration of NK cells adjuvant therapy in combination with surgical treatment and chemotherapy on in patient with advanced gastric signet-ring cell carcinoma. The treatment decisions were fully compliant with the patient' choices, better reflecting practical clinical situations. NK cells infusion combined with surgical and chemotherapy was well tolerated and showed great potential for the prevention of gastric signet-ring cell carcinoma recurrence and prolonging of survival.

Abbreviations

GC: gastric cancer; SRC: signet-ring cell; CEA: carcino-embryonic antigen; CA: carbohydrate antigen; AFP: alpha fetoprotein; NSE: neuron-specific enolase; HCG-BET: human chorionic gonadotropin; UCB: umbilical cord blood; MNC: mononuclear cells; NK: Natural killer; HER-1: human epidermal growth factor receptor-1; HER-2: human epidermal growth factor receptor-2

Declarations

Consent

We provided precise explanations of the therapy to the patient. She gave written informed consent for the publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests

The authors have no financial or personal relationships with people or organizations that could inappropriately influence this work.

Funding

There was no funding body.

Availability of data and materials

Not applicable

Ethics approval and consent to participate

Not applicable

Consent for publication

Informed consent was obtained from the patient.

Authors' contributions

Wenzhuo Yang and Chuntao Wu conceived and designed the study and collected, assembled, analyzed, and interpreted the data. Zhengyang Sun and Zhongbo Wang prepared the NK cells. Yuanyuan Jin and Zhaoyong Yang wrote the manuscript and approved the final manuscript. All authors read and approved the final manuscript.

Acknowledgements

We acknowledge the patient who agreed to participate in this study. This study received no financial support.

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Figures

Timeline

Figure 1

Schematic representation of the clinical history, therapy, and visits of a patient diagnosed with gastric signet-ring cell carcinoma (stage IIIC, T4a, N3a, M0) in December, 2015 (V0). The patient was treated with oxaliplatin and tegafur combined chemotherapy for six cycles after the radical gastrectomy during the period of December 2015 to May 2016 (V1). The level of CA72-4 decreased significantly after the treatment (V2). Three months later (V3), the level of CA72-4 increased to 86.34 U/mL rapidly. On November, 2016 (V4), the patient began to receive NK cell treatment on a 3-yearly basis. The detection of tumor markers and the abdominal CT scan after NK therapy (V4–V8) revealed that it was becoming normal and in good condition.

Figure 2

Dot plots of percentages of CD3+T and NK cells in peripheral blood of the patient by flow cytometry analysis