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Research paper

Tumor cryoablation in combination with natural killer cells therapy and Herceptin in patients with HER2-overexpressing recurrent breast cancer

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ABSTRACT

In this study, we investigated the clinical benefits of a combination of tumor cryoablation with natural killer (NK) cells therapy and Herceptin for human epidermal growth factor (HER) 2-overexpressing recurrent breast cancer. From May 2015 to May 2016, 48 patients who met the enrollment criteria were assigned to three groups (n = 16): cryoablation group (group I), cryoablation-NK cells therapy group (group II) and cryoablation-NK cells therapy-Herceptin group (group III). Safety and short-term effects were evaluated. All the adverse effects were manageable and acceptable. The three-therapy combination treatment not only yielded good clinical efficacy, it also improved the quality of life; reduced levels of circulating tumor cells (CTCs); reduced carcino-embryonic antigen (CEA) and cancer antigen 15-3 (CA15-3) expression; enhanced immune function significantly. Furthermore, it can resulte in significant prolongation of progression free survival (PFS). This is the first clinical study to demonstrate the benefit of the three-therapy combination of tumor cryoablation, NK cells therapy, and Herceptin for HER2-overexpressing recurrent breast cancer.

1. Introduction

In the USA, the incidence of various types of breast cancer has increased by 2.5% over the past decade, and it has become the most common malignancy affecting the health of women in that country (DeSantis et al., 2014; Toothaker and Rubin, 2009). Despite advances in surgical and catheter intervention techniques, approximately one-third of breast cancer patients experience relapse or metastasis within 5 years (Jemal et al., 2011).

Human epidermal growth factor (HER)2-overexpressing occurs in 25–30% of case of recurrent breast cancer and leads to a particularly aggressive form of the disease (Slamon et al., 1987). As it cannot be cured, the treatment goal in such cases is to control the disease symptoms, relieve pain, and prolong survival to the greatest extent possible. Chemotherapy plays an important part in the treatment of HER2-overexpressing breast cancer (Smith et al., 2002), but the related toxicity and side-effects may influence the health and quality of life (QOL) of patients (Broeckel et al., 1998; Chia et al., 2007). Hence, more effective and safer treatments must be identified to improve the survival and patients of QOL of HER2-overexpressing recurrent breast cancer.

Tumor cryoablation is a new surgical technique wherein tumors are frozen and destroyed using ultra-low temperature cryoprobes. This method has been successfully used for the ablation of tumors in the liver (Hinshaw and Lee, 2007; Schuld et al., 2014), lung (Niu et al., 2013a; Zhikai et al., 2013), and kidney (Kapoor et al., 2013; Rukstalis et al., 2001). A long-term follow-up study of tumors treated with cryoablation showed that it can serve as an important option for a wide range of unresectable cancers and could yield long-term survival (Orlacchio et al., 2008; Xu et al., 2003). Therefore, cryoablation is a feasible option for the treatment of HER2-overexpressing recurrent breast cancer (Tarkowski and Rzaca, 2014).

Manipulation of the immune system for therapeutic benefit in breast cancer patients has been studied for several decades (Hadden, 1999; Kubo et al., 2003; Spellman and Tang, 2016). Natural killer (NK) cells are important components of the innate immune system and have a critical role in the early host defense against cancer (Cheng et al., 2013; Zhao et al., 2015). With advancements in the NK cells biology field and enhancements in our understanding of NK function, NK cells has become a powerful immunotherapy tool for breast cancer treatment (Roberti et al., 2012).

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The HER2 protein is a 185-kD transmembrane tyrosine kinase with homology to the epidermal growth factor receptor (Montemurro et al., 2004). Trastuzumab (Herceptin) is a humanized monoclonal antibody with specificity for the HER2 protein. Herceptin is considered to be an effective therapeutic approach against HER2-overexpressing breast cancer. It has been shown to induce tumor regression in up to 20% of heavily pretreated metastatic breast cancer (Baselga et al., 1996).

On the basis of the studies mentioned above, we conducted a randomized, single-blind, single-center clinical study to investigate the efficacy and safety of combinations of cryoablation, NK cells therapy, and Herceptin for HER2-overexpressing recurrent breast cancer. We focused on a preliminary exploration for clinical practice, and then on future perspectives in the treatment of HER2-overexpressing breast cancer.

2. Methods

2.1. Ethics

This study was registered in ClinicalTrials.gov (ID: NCT02844335; Ph1/Ph2) and approved by the Ethics Committee of Guangzhou Fuda Cancer Hospital (Guangzhou, China). Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki. All the participants were recruited and treated at Guangzhou Fuda Cancer Hospital.

2.2. Patient eligibility

Women with histologically confirmed, bidimensionally measurable, HER2- overexpressing recurrent breast cancer, were eligible for this study. HER2 assessment was undertaken on formalin-fixed paraffin-embedded tumor sections by sequential immunohistochemical analyse with two murine monoclonal antibodies: 4D5 and CB11. HER2 status was defined as “positive” if weak to moderate (2+) or complete (3+) membrane staining in > 10% of tumor cells with either antibody was observed.

Women were required to be between 20 and 70 years of age. The ideal candidates for this study were those with: a lifespan > 6 months; Karnofsky performance status (KPS) score ≥60; platelet count $\geq 80 \times 10^9$/L; white blood cell count $\geq 3 \times 10^9$/L; neutrophil count $\geq 2 \times 10^9$/L; hemoglobin ≥90 g/L; prothrombin time international normalized ratio ≥1.5; an absence of level 3 hypertension, severe coronary disease, myelosuppression, respiratory disease, and acute or chronic infection; adequate hepatic function (bilirubin < 30 μmol/L, aminotransferase < 60 U/L) and renal function (serum creatinine < 130 μmol/L, serum urea < 10 mmol/L).

Patients were divided randomly into three groups. Group I was treated with cryoablation, group II was treated with cryoablation-NK cells therapy, and group III was treated with cryoablation-NK cells therapy-Herceptin. The patient classification is listed in Table 1.

Table 1
Patient characteristics.

Characteristic	Group I (n = 16)	Group II (n = 16)	Group III (n = 16)	*P* value
Age (years)				*P* = 0.557
Median age (range)	54 (29–68)	53 (26–71)	57 (31–69)	
< 50	7	10	8	
≥ 50	9	6	8	
Estrogen receptor status				*P* = 0.519
Positive	5	7	4	
Negative	11	9	12	
Progesterone receptor status				*P* = 0.776
Positive	7	6	8	
Negative	9	10	8	
HER2 expression status				*P* = 0.312
IHC 2+	5	3	7	
IHC 3+	11	13	9	
Tumor histology				*P* = 0.772
Invasive ductal carcinoma	13	12	10	
Invasive lobular carcinoma	1	2	2	
Other (mixed, mucinous, metaplastic, apocrine)	2	2	4	
Previous therapy				*P* = 0.993
Surgery	14	13	13	
Chemotherapy	11	9	10	
Radiotherapy	7	8	7	
Sites of metastasis				*P* = 0.875
Lymph node	9	11	7	
Lung	7	9	8	
Bone	6	4	7	
Liver	3	6	4	
Clinical stage (AJCC)				*P* = 0.766
III	10	11	9	
IV	6	5	7	
KPS				*P* = 0.853
60	4	3	4	
70	4	2	3	
80	8	11	9	

AJCC, American Joint Committee on Cancer staging system; KPS, Karnofsky performance status.

2.3. Cryoablation procedure

Tumor cryoablation was performed according to a protocol published previously (Li et al., 2014; Niu et al., 2013b). The percutaneous puncture point was determined using computed tomography (CT). Cryoablations were undertaken using the commercially available Cryocare Surgical System (Endocare, Irvine, CA, USA) with argon and helium gas as the cryogen. Cryoprobes (1.7 mm) were inserted into the center of the tumor under CT guidance. Two freeze-thaw cycles were performed, wherein a temperature between −125 °C and −150 °C was reached and maintained for 15 min, followed by rewarming at 15–20 °C. A margin of at least 1 cm of normal breast tissue was also frozen circumferentially around the tumor. For masses > 5 cm in diameter, two or three cryoprobes were placed within the center and periphery of the tumor to ensure freezing of the entire mass. After cryoablation, an antibiotic (cefoperazone) and haemostatic agent (hemocoagulase) were administered, and vital signs (blood pressure, pulse, respiration, oxygen saturation of blood) were monitored routinely.

2.4. NK cell therapy

NK cells were generated according to protocols published previously in good manufacturing practice (GMP) condition (Zhang et al., 2010). In brief, 80 mL of peripheral blood from allogenic donors was drawn 7 days before cryoablation, and immunotherapy was administered 3–5 days after cryoablation (adoptive transfer of NK cells was performed twice continuously). To ensure appropriate selection of donors, the killer cell immunoglobulin-like receptor (KIR) genotype should not match with the human leukocyte antigen (HLA) class I molecules of the patient (Forte et al., 2009; Kunert et al., 2007; Moretta and Moretta, 2004; Witt and Christiansen, 2006; Zhang et al., 2010). The peripheral blood from allogenic donors and recipients was tested with the TIANamp Blood DNA kit (Tiangen Biotech, Beijing, China) and KIR/HLA-Cw Genotyping Low Resolution kit (PCR-SSP) (Super Biotechnology Developing, Tianjin, China).

For NK cell culture, we used a Human HANK Cell *In Vitro* Preparation kit (Hank Bioengineering, Shenzhen, China), along with