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Trastuzumab decreases the number of circulating and disseminated tumor cells despite trastuzumab resistance of the primary tumor

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Abstract

We have recently shown that despite of the fact that the ErbB2-positive JIMT-1 human breast cancer cells intrinsically resistant to trastuzumab *in vitro*, trastuzumab inhibited the outgrowth of early phase JIMT-1 xenografts in SCID mice via antibody-dependent cellular cytotoxicity (ADCC). Here we show that trastuzumab significantly reduces the number of circulating and disseminated tumor cells (CTCs and DTCs) in this xenograft model system at a time when the primary tumor is already unresponsive to trastuzumab. This observation suggests that ErbB2 positive CTCs and DTCs might be sensitive to trastuzumab-mediated ADCC even if when the primary tumor is already non-responsive. Thus, trastuzumab treatment might also be beneficial in the case of patients with breast cancer that is already trastuzumab resistant.

Keywords: Circulating tumor cells; Disseminated tumor cells; Trastuzumab resistance; ErbB2/HER2; Breast tumor xenografts

1. Introduction

Death of most cancer patients is caused by metastatic spread of cancer cells from the primary tumor to distant organ. It has been suggested that approximately 10^6 tumor cells per gram of tumor tissue per day shed from the primary tumor into the bloodstream [1]. Escape of tumor cells occurs primarily across the wall of mosaic tumor blood vessels composed of endothelial and tumor cells. Chang et al. suggested that approximately half of the tumor cells contacting the vessel lumen is shed in a day [2].

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Although a great number of tumor cells move to the circulation and become circulating tumor cells[1] (CTC) [3], only a minority of them can form metastasis. Luzzi et al. showed that the formation of metastasis is a highly inefficient process: only 0.02% of CTCs is able to form metastasis [3]. In the bloodstream a large fraction of the CTCs die quickly due to killing by the immune system [4,5], hemodynamic forces [6] or apoptosis evoked by loss of cell attachment and cell–matrix connections [3]. Some CTCs extravasate into distant organs and persist there as disseminated tumor cells (DTC), which may be dormant and so partially resistant to cytotoxic chemotherapeutic agents [7–9]. A very small proportion of extravasated CTCs (2%) is capable of dividing and forming micrometastases,[1] and an even smaller fraction (0.02%) can evolve to a full-fledged metastasis [3,9]. A circulating multicellular tumor aggregate (CMTA) is a group of attached cells shed from the primary tumor, which reach the circulation and migrate collectively. The presence of CMTAs in the blood is a marker of high metastatic potential [10,11].

Although metastasis formation from CTCs and DTCs is a highly inefficient process, the chance of metastasis formation can increase, since breast tumor size reach a typical clinically detectable size of 2–3 cm (diameter) in 1–3 years, as shown by studies analyzing serial screening mammograms [12,13]. The presence of CTCs in primary and metastatic breast cancer is associated with poor prognosis [14–16]. It has been published that ~50% of metastatic breast cancer patients had more than five CTCs in 7.5 ml blood; the presence of more than five CTCs was a strong independent predictor of poor outcome [14]. Bone marrow micrometastases were detected in 30% of stage I–III breast cancer patients, who had larger and higher-grade tumors, and more often had lymph node metastases. The presence of micrometastases was an independent predictor of poor outcome [17].

The overexpression of ErbB2 (HER2) (a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases) occurs in 20–30% of invasive breast cancers [18–20] and is associated with poor prognosis and rapid relapse [21]. ErbB2-positive circulating tumor cells indicate poor clinical outcome in stage I to III breast cancer patients. The presence of ErbB2-positive CTCs has been associated with larger tumor size, negative estrogen receptor (ER) status, poor histological differentiation, and lymphovascular invasion [22]. Interestingly, it has been reported that nearly one-third of patients whose primary tumors were negative for ErbB2 had CTCs with amplified ErbB2 (determined by FISH) [23,24]. In addition, ErbB2-positive DTCs can be detected in patients with ErbB2 negative primary tumors (determined by immunohistochemistry) [25]. Wülfing et al. observed a discrepancy between the detection of ErbB2-positive CTCs and the ErbB2 score of the corresponding primary tumor [22]. Furthermore, no correlation was found between the ErbB2 staining score of the primary tumor and the presence of ErbB2-positive micrometastases in the bone marrow [26,27].

Trastuzumab (Herceptin®) (a recombinant humanized monoclonal antibody against the extracellular domain of ErbB2) has a remarkable antitumor effect, and is now widely used for the treatment of breast cancer [28,29]. After initial usage in metastatic breast cancer patients, subsequent studies of adjuvant trastuzumab therapy showed significant benefit in primary breast cancer as well [30,31]. It has also been shown that trastuzumab reduced the number of CTCs and bone marrow micrometastases in chemotherapy-resistant breast cancer patients [32].

We previously showed that JIMT-1 breast cancer cells are intrinsically resistant to trastuzumab *in vitro*, furthermore, in a mouse xenograft model trastuzumab caused a significantly inhibited outgrowth of very small early phase JIMT-1 xenografts via trastuzumab-mediated antibody-dependent cellular cytotoxicity (ADCC) [33]. In this study, we have investigated the effect of trastuzumab therapy on the number of CTCs, DTCs and micrometastases in our breast cancer xenograft model system and have shown that trastuzumab decreases the number of circulating and disseminated tumor cells despite trastuzumab resistance of the primary tumor.

2. Materials and methods

2.1. Cell line

JIMT-1, an ErbB2 positive human breast cancer cell line, was grown in Ham's F-12/DMEM (1:1) supplemented with 10% FCS, 60 U/L insulin and antibiotics [34]. The JIMT-1 cell line is avaible from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (www.dsmz.de).

[1] We use the terminology suggested by Paterlini-Brechot and Benali [3].