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Original article

Moderate intermittent negative pressure increases invasiveness of MDA-MB-231 triple negative breast cancer cells

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ABSTRACT

Background: To investigate the effect moderate intermittent negative pressure breast reconstructive model exerts on human triple negative breast cancer cell (TNBC) invasion and explore the related mechanism.

Methods: The human TNBC cell line MDA-MB-231 was used. Cells in external volume expansion (EVE) group were exposed to an intermittent −25 mmHg for 12 h; the pressure for non-EVE group was constantly 0 mmHg. In vivo, MDA-MB-231 cell suspensions were injected subcutaneously into dorsal skin of nude mice (n = 27 mice/group). Tumors on mice in EVE group received −25 mmHg suction 3 h/day; while mice in non-EVE group were under normal pressure. Cell invasion assay, ELISA, RT-PCR, western blot analysis and immunohistochemistry were used to evaluate the inflammation, epithelial-mesenchymal transition (EMT) and angiogenesis between the two groups in both vitro and vivo experiments.

Results: MDA-MB-231 cells in the EVE group were more invasive and had higher expressions of IL-8 (30.02 ± 10.44 pg/ml vs. 18.82 ± 9.26 pg/ml, $P < 0.05$) and TNF-α (20.59 ± 4.72 pg/ml vs. 14.10 ± 3.36 pg/ml, $P < 0.05$) than the non-EVE group. Grafted MDA-MB-231 tumors in EVE group showed a more obvious epithelial-mesenchymal transition at 2 week and better angiogenesis at 2 and 4 week, respectively.

Conclusion: Moderate intermittent negative pressure induces MDA-MB-231 cells to be more invasive. Future studies should figure out other effects this intervention may bring. Clinical studies should also be conducted to further evaluate its safety and optimize the clinical model.

1. Introduction

As an important social psychological indicator to evaluate the quality of life on breast cancer survivors after receiving mastectomy or lumpectomy, the demand for breast reconstruction have constantly increased over the last several years [1–3]. Compared with the traditional flap-based reconstruction, the new alternatives tend to be more minimally invasive and convenient for nursing. Among them, fat grafting facilitated with the negative pressure external volume expansion (FGNP-EVE) gains impressive clinical results [4,5] (Fig. 1). By optimizing the recipient site [6], better volume retention could be achieved than simply filling the grafted fat [7]. The negative pressure stimulation is considered to be able to provide a more suitable recipient bed [6] for fat grafting and induce adipogenesis [8], which has been shown in murine model experiment. Considering of the present clinical and experimental evidences, it tends to become a common practice to suggest patients to use the commercial external volume expansion device on the recipient site for several hours per day before or after fat grafting, it is where the definition of moderate intermittent negative pressure originates from in this study.

Similar to fat transferring, although it acquires good aesthetic outcome, the combinative method is also questioned for the potential risk of increasing local tumor recurrence, invasion and

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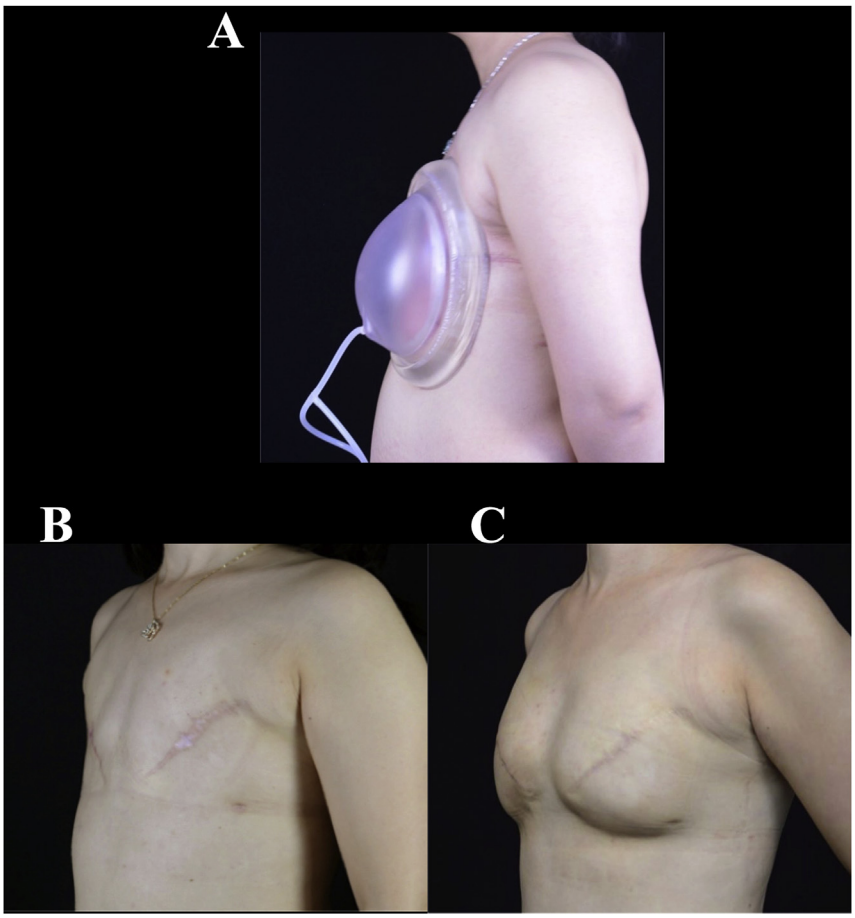

Fig. 1. A 28-year-old woman who received fat grafting facilitated with the negative pressure external volume expansion five years after receiving bilateral mastectomy because of suffering breast cancer (a) Clinical use of the negative pressure suction device. (b) Pre-operative photo. (c) Post-operative photo.

metastasis to the patients who have breast cancer history. So far, the current conclusion on fat transfer safety evaluation has shown no association with higher clinical recurrence in some certain type of breast cancer [9]. However, it still needs to be further investigated due to insufficient follow-up time, the lack of matched groups, and bias [10]. Meanwhile, there is a remarkable contradiction between experimental and clinical findings, the related experimental studies have demonstrated some evidences on its promotion on breast cancer progression both in vitro and vivo [11–14]. Compared with the inconsistency between fat grafting related clinical and experimental oncological safety assessments, the safety evaluation on negative pressure suction is limited. Previous relevant reports are mainly focused on the effects of intensive negative pressure on malignant wounds [15]. Also, stimulating modulations in these studies are not much comparable with clinical application of the negative pressure suction device [8]. Since invasiveness is the biological characteristic which is close related to metastasis and prognosis, we firstly designed this study to further observe if moderate intermittent negative pressure, which has been used in clinical practices, would influence the invasiveness of breast cancer cells.

In order to preliminarily investigate the effect moderate intermittent negative pressure exerts on breast cancer cell invasion ability and explore the related mechanism, triple negative breast cancer (TNBC) cell line MDA-MB-231 was used. In this study, a chamber with moderate intermittent negative pressure inside and an animal model similar to clinical suction one were manufactured. The change of invasiveness and related mechanisms were explored both in vitro and vivo.

2. Methods

2.1. Device

An enclosure chamber made from polypropylene was manufactured and put in the incubator as it shown in Fig. 2 for cell culturing. By keeping balance the inflow and outflow, the pressure in the chamber stays at −25 mmHg [6,8] which was in the range used by the clinical external volume expansion during working hours. After 12 working hours, gas in the box and incubator

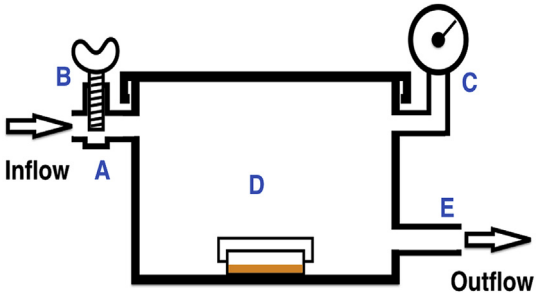

Fig. 2. In vitro cell culture system (A) Inflowing vent (B) Control valve (C) Pressure measurer (D) Cell culture dish (E) Outflowing vent.