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Denervation leads to volume regression in breast cancer

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Summary The nervous system plays a key role in controlling the dynamic functions of multicellular complex organisms. Although peripheral nerves are supposed to play a pivotal role in tumor growth and dissemination, little experimental evidence exists to date. We assessed the effect of denervation on breast cancer growth by magnetic resonance imaging (MRI) in rats. Human breast cancer cells were implanted into adipofascial flaps with intact or surgically excised supplying nerve. Tumor volumes were measured 2 and 8 weeks after implantation by *in vivo* MRI. Results were validated by histology. Postoperative tumor volumes at 2 and 8 weeks were reduced by 76% (95% CI: 22–93%) in the denervated groups. Tumor area as determined histologically was reduced by 70% (95% CI: 60–78%). Thus, peripheral denervation may be an

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effective surgical approach for the palliative treatment of locally progressing or uncontrollable breast cancer.

Introduction

Breast cancer is still one of the most prevalent causes of death in females. Despite considerable improvements in early diagnosis and new treatments over the past few decades,[1,2] some patients still have poor outcome and develop unresectable tumors.[3] Thus, there is a need for alternative therapeutic options, especially for locally progressing, uncontrollable cancer types. The frequency of locally advanced breast cancer has diminished greatly because of screening mammography and early detection.[4] The incidence among white Americans has been reported to be 2.1%.[4]

Tumor development can be regarded as a result of constant mutual interactions between tumor cells and their surrounding microenvironment.[5] The neuronal influence on tumor biology was initially described decades ago, but little is known about the potential biological effects of "neoneurogenesis".[6] Possibly, cancer cells can take the advantage of the factors released by the nerve fibers to create a positive microenvironment for proliferation and cell survival.[6]

In addition to the release of angiogenic and lymphangiogenic factors, tumors secrete neurogenic factors and axon guidance molecules.[7] Furthermore, there are several lines of evidence that show an innervation of tumors and a correlation between the detection of nerve cell markers in tumors and a poorer outcome of the cancer disease.[8,9] Moreover, researchers have postulated a crosstalk between cancer cells and nerve fibers as a strategy for survival.[6] The communication between the tumor cells and their microenvironment is the driving force behind the process of tumor progression, as cancer cells can use factors, released by their innervating nerve fibers, to generate a stimulating microenvironment for their own survival and proliferation.[10,11] On one side, tumor cells stimulate their own innervation, and on the other hand, these innervating nerve endings provide neurotransmitters that facilitate metastasis formation and may influence other tumor cell functions such as their proliferative activity.[6,10–14] Tumor cell migration along neuronal processes (perineural invasion) correlates with poor prognosis in many cancer types.[8,9] Interestingly, it has been reported that neural-related factors are important players in cancer cells.[15] Moreover, it has been suggested that neural-related factors establish a direct connection between the nervous system and tumor cells.[15]

Combining these arguments, a hypothesis was created that tumors are capable of stimulating their own innervation in a process similar to angiogenesis and lymphangiogenesis. This process was named "neoneurogenesis".[16] However, the potential role of innervation in breast cancer development has not been tested in an experimental *in vivo* setting. Thus, our hypothesis was that breast cancer growth can be reduced by denervation at the time of tumor cell implantation.

Materials and methods

Pilot study

This study was approved by the ethical commission of the University of Basel and the veterinary institute. To optimize the ectopic tumor induction, we tested three different breast cancer cell lines (MDA-MB 231, MDA- MB 453, and MDA-MB468) in rats with three animals per group. MDA-MB 231, MDA-MB 453, and MDA-MB 468 were obtained from the cell line bank of Friedrich Miescher Institute Basel, Switzerland. Unless otherwise stated, all other reagents were obtained from Sigma Aldrich, Switzerland. The goal was to find a cell line that could induce a reproducible and on MRI measurable tumor within 2 weeks after cell implantation, but one that does not exponentially grow after week 2 to avoid early euthanasia of experimental animals. Rats were euthanized after 2 and 8 weeks, respectively, and all experiments were performed in triplicate; the data are representative of multiple experiments.

Cell culture

Cells were pelleted by centrifugation at 835 × g for 15 min at 4 °C, washed in Hank's buffered saline solution, frozen in a viable state in DMSO Freeze media (Invitrogen; Gaithersburg, MD), and stored in liquid nitrogen until used. Frozen cells were maintained in DMEM/F12 (Invitrogen) supplemented with 5% heat-inactivated horse serum (Gemini BioProducts, W. Sacramento, CA) and 100 units/ml penicillin–streptomycin. Cells were passaged by trypsinization. All cells were incubated in a 5% carbon dioxide atmosphere at 37 °C. The culture medium was changed every second day, and cells were passaged every 2–4 days using accutase (PAA Laboratories GmbH, Linz, Austria).

Main study

Surgical technique

Ten-week-old female Sprague-Dawley (SD) rats (24 rats weighing 200–250 g (Harlam, The Netherlands)) were used in this study. A sterile surgical surface was prepared, and anesthesia was induced using isoflurane (Attane) at 5% concentration. An appropriate depth of anesthesia was confirmed using the tail pinch test. Isoflurane percentage was diminished then during the surgery to approximately 2–3%, and the heart rate and breathing were monitored. All surgical procedures were performed under an operating microscope (Carl Zeiss, Germany). The left lower abdomen, around the abdominal breast gland, was shaved, and the skin was disinfected using Betadine®.