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Brachytherapy ■ (2018) ■

BRACHYTHERAPY

Iodine-125 seed brachytherapy inhibits non−small cell lung cancer by suppressing epithelial-mesenchymal transition

Yana He[1,2,1], Liang Li[1,1], Jihua Liu[2], Xuening Zhang[1,*]

[1]*Department of Medical Imaging, Second Hospital of Tianjin Medical University, Tianjin, China*

[2]*Department of Medical Imaging, The First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, China*

ABSTRACT

PURPOSE: Metastatic non−small cell lung cancer (NSCLC) has posed a great clinical challenge with high mortality. Iodine-125 (I-125) seed brachytherapy has not been widely applied in clinic against NSCLC.

METHODS AND MATERIALS: Mice were injected with human H23 NSCLC cells to establish a mouse xenograft model, which received I-125 seed implantation. The curative effect, pathological impairments, and survival rate of mice were investigated. Changes in the expression levels of epithelial-mesenchymal transition (EMT) markers, including N-cadherin, E-cadherin, and vimentin, in the xenograft tumors were analyzed using reverse transcription polymerase chain reaction and Western blot.

RESULTS: The tumor volume and pathological effect were reduced by I-125 seed implant. I-125 seed implant also significantly improved survival rate of the model mice. Expression patterns of N-cadherin, E-cadherin, and vimentin were reversed in I-125 seed−implanted mice in comparison with control mice, indicating suppressed EMT.

CONCLUSIONS: I-125 seed brachytherapy significantly inhibits NSCLC by suppressing EMT in a mouse model. © 2018 Published by Elsevier Inc. on behalf of American Brachytherapy Society.

Keywords: Iodine-125; Brachytherapy; Epithelial-mesenchymal transition; Non−small cell lung cancer

Introduction

In spite of the fast progress of surgical procedure and drug development, lung cancer is still one of the top causes of cancer-related death around the world [1], with an overall 5-year survival rate of approximately 15% [1]. Surgery is now regarded as the best option of treatment for non−small cell lung cancer (NSCLC). However, it is often not until advanced stage that lung cancer is diagnosed, and no more than 25% of patients with NSCLC are suitable for surgical procedures [2]. An alternative therapeutic strategy for treating cancer is chemotherapy [2], but it is often unsuccessful to eliminate all the tumor cells as a result of drug resistance. Some patients are intrinsically resistant to chemotherapy, which is referred as intrinsic resistance, whereas other patients, although initially sensitive to chemotherapy, develop acquired resistance eventually even after combination therapy. Therefore, therapeutic resistance and metastasis are the major reasons for failures of cancer treatments.

Epithelial-mesenchymal transition (EMT) is the evolutionarily conserved process of epithelial cells converting to mesenchymal cells [3], which was initially described in the research of embryonic development. EMT is demonstrated to play essential roles for embryonic development, neural nest, gastrulation, as well as development of heart and other tissues or organs [4]. Recent studies have shown that EMT is associated with pathogenesis of various types of cancer, including lung cancer [5]. Cells lose or redistribute epithelial proteins during EMT and obtain mesenchymal proteins, leading to the loss of epithelial polarity and acquisition of a fibroblastic phenotype that is highly motile. Such cells can digest through the basement membrane [6−8] and activate tumor-specific gene program [3, 9]. Upregulation of genes during EMT is associated with poorly differentiated tumors relative to low-grade tumors

Received 5 February 2018; received in revised form 28 February 2018; accepted 12 March 2018.

Financial disclosure: The authors declared no financial disclosures.

Conflict of interest: The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

* Corresponding author. Department of Medical Imaging, Second Hospital of Tianjin Medical University, 23 Pingjiang Road, Hexi District, Tianjin 300211, China. Tel./fax: 86-18622662930.

E-mail address: zhangxn2266@163.com (X. Zhang).

[1] Contributed equally.

1538-4721/$ - see front matter © 2018 Published by Elsevier Inc. on behalf of American Brachytherapy Society.
https://doi.org/10.1016/j.brachy.2018.03.002

[10]. Switching from E-cadherin to N-cadherin exhibited strong and significant correlation with progression of cancer [11]. EMT is shown to be involved in several epithelial cancers, and epithelial cancers indeed contribute to the majority of human malignancies [12]. Hence, EMT is a critical event in the progression, invasion, and metastasis of carcinomas and generally related to a particularly dismal prognosis [6, 8, 12].

Brachytherapy refers to a form of radiotherapy in which sealed sources of radioactive material are permanently inserted directly into tumors or into body cavities. It has been applied with success in the treatment of inoperable solitary lung cancers, with minimum radiation exposure to surrounding tissues [13−15]. Iodine-125 (I-125) seed implantation has recently attracted increasing attention as an efficient brachytherapy technique due to advantages such as (1) percutaneous implantation under the guidance of CT or ultrasound, (2) effective dose of irradiation applied in one procedure, (3) reduced radiation damage outside the targeted tumor, and (4) extended period to eliminate tumor [16, 17]. Because it is able to provide high precision, strong lethality, fewer complications with little trauma, I-125 radioactive seed implantation is now widely used in clinical practice for treating tumor. However, it is not yet widely applied in the treatment of NSCLC, and the signaling pathways involved in the efficacy of I-125 seed brachytherapy on NSCLC has not been well investigated.

In this study, we established a mouse model of NSCLC xenograft tumors, to investigate the effect of I-125 seed implant on the *in vivo* growth of these tumors. Furthermore, we also aimed to uncover the mechanism underlying the antitumor effect of I-125 seed implant.

Methods and materials

Radiation source and reagents

Brachytherapy seeds I-125 and instrument for the I-125 seed implantation were purchased from Ningbo Junan Pharmaceutical Technology Company (Ningbo, China). A single seed is 0.84 mm in diameter and 4.5 mm in length; two different apparent radioactivities were included: 0.4 mCi/seed and 0.8 mCi/seed, and the half-life was 59.43 days. The I-125 seeds were randomly selected for testing of activity to confirm the integrity of seed container and the apparent activity of the seeds. The 0.4 mCi/seed was found to be less toxic to the physical condition of animals and therefore used in the present study.

Establishment of the animal models

All the animal experiments were approved by the Ethics Committee of Second Hospital of Tianjin Medical University. Thirty BALB/c nude mice (4−6 weeks old; 20.0−24.0 g) were used for the experiments. Mice were allowed to acclimatize for at least 48 h before experimentation and fed with a standard rodent chow.

The human H23 NSCLC cell line was purchased from the American Type Culture Collection (Manassas, VA). For preparation of H23 cell suspension, the human H23 NSCLC cells were administered into the abdominal cavity of a nude mouse to culture, and the carcinomatous ascites developed rapidly after injection. To minimize the potential differences in tumor growth, H23 tumor cell suspension was harvested from the same tumor donor mouse. The suspension was mechanically homogenized and adjusted to a concentration of 7×10^6 viable cells per milliliter.

To establish a rodent model, 0.2 mL of H23 tumor cell suspension was injected subcutaneously into the right axillary space of a mouse with an 18G needle. Using B-ultrasound to monitor the position of inoculation in each mouse, we selected mice with just one tumor in the armpit of the right forelimb. Treatments began 14−16 days following the implantation. Mice with tumor diameter of ~1.0 cm and above were chosen and this day was defined as "Day 0".

Grouping and I-125 seeds irradiation

Of the 30 mice, 24 (tumor diameter reached 1.0 cm) were divided randomly into two groups, 12 in each group, followed by B-ultrasound-guided I-125 seed brachytherapy. Mice in the control group were not operated, whereas the I-125 group of mice was treated with 0.4-mCi I-125 seed implant. All brachytherapy seeds were inserted into the same location.

Physical condition assessment of model animals

All mice were marked and continuously observed for 28 days, the numbers of alive mice in each group were recorded, and reactions of tumor and its surrounding skin were noted. The size of tumors was measured every 4 days with calipers during the study period, and the tumor volume and the tumor inhibit rate were calculated. The tumor volume was calculated using the following formula: $V = (A \times B^2)/2$, where V is tumor volume, A is the tumor measurement at the longest point, and B is the tumor dimension at the widest diameter. The tumor inhibit rate (R) was calculated using the following formula: $R = (V1 - V2)/V1 \times 100\%$, where V1 is the average tumor volume of the control group, and V2 is the average tumor volume of the treatment group. Each mouse was weighed at the time of administration. At 28 days after implantation, animals were sacrificed under anesthetized condition, and the tumor mass was collected and weighed and then prepared for pathological evaluations.

Pathological assessment

After 28 days of treatment, animals were sacrificed under anesthetized condition by cervical dislocation for pathological assessment. Two experienced researchers