

Establishment and Preliminary Application of Axillary Lymph Node Metastasis Model in Breast Cancer Transplantation

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Abstract

Lymph node is essential to improve the quality of life and extend the survival of patients. In this study, an animal model of axillary lymph node metastasis of rabbit breast transplanted tumour was established by the tumour embedding method to explore the feasibility of the new MR lymphoid targeting contrast agent HD120-GD-DTPA showing the metastatic lymph nodes and implanting the radioactive 125I particles into the lymph node to treat the metastatic lymph nodes. The results showed that the enlarged lymph nodes were metastatic ones. The enhancement scan showed: The difference of the lymph nodes sizes between the control group and the test group was statistically significant ($P < 0.01$). Therefore, the lymph node metastasis is the most common route of metastasis, so axillary lymph node status and its treatment are important. The new lymphoid targeting contrast agent HD120-GD-DTPA can be used for lymph node imaging and identification of the benign or malignant nature of lymph nodes. 125I can effectively inhibit the growth of metastatic lymph nodes, and it is expected to be used clinically.

Objective

Lymph node metastasis is one of the most important reasons which cause death in breast cancer patients, and the common metastatic site is the axillary lymph nodes. Therefore, detecting the invasive lymph nodes earlier is essential to improve the quality of life and extend the survival of patients. An animal model can provide an important tool to screen the related transfer genes, study the molecular mechanisms of lymph node metastasis, and explore the treatment of diseases. In this study, an animal model of axillary lymph node metastasis of rabbit breast transplanted tumour was established by the tumour embedding method to explore the feasibility of the new MR lymphoid target-

ing contrast agent HD120-GD-DTPA showing the metastatic lymph nodes and implanting the radioactive 125I particles into the lymph node to treat the metastatic lymph nodes.

Methods

Selecting 40 healthy New Zealand white rabbits randomly, quality was 3.0-3.5kg. Thirty randomly selected rabbits were used to establish the axillary lymph node metastasis model of breast transplanted tumour by tissue block embedding method. The VX2 cancer tissue was planted into the third breast pad of the rabbit on the left side. Then detecting the growth status of the orthopaedic and axillary lymph nodes



by palpation and ultrasonography. Removing the breast transplanted tumour tissue after being able to touch the enlarged lymph nodes (reversing the nipple). The size of metastatic lymph nodes was measured by the ultrasound diagnostic apparatus weekly. When the maximum diameter of the lymph nodes was about 10mm, twenty rabbits were divided into experimental and control groups (selecting 10 rabbits randomly from the 30 rabbits and the remaining healthy 10 rabbits) and then do the MR examination, using 3D TOF CE-MRA sequence scan firstly, then injecting the new lymphoid targeting contrast agent HD120-GD-DTPA 0.1 ml at the left side of the third areola around 3, 6, 9oclock direction. Finally, doing the enhanced MR scan. The time of scanning was 30 min, 60min, 90min and 120min after injection, Plain scan and enhanced scan parameters are the same. After scanning, the signal intensity of the two groups of lymph nodes were measured by DICOM software and the normalized signal intensity (SI) and the enhancement rate (En%) were calculated. Comparing the enhancement degree of the lymph nodes in different periods and the characteristics of strengthening. After two days, 19 rabbits selected from the animal model were randomly divided into experimental group (A=10) and control group (B=9). The experimental group of metastatic lymph nodes were implanted with radioactive 125I particles, the control group of lymph nodes were implanted with no radioactive 125I particles. The diameter of the lymph nodes was measured by ultrasonography. The enlarged lymph nodes at the axilla of the remaining animal model were fixed and embedded, and routine HE staining. Observing the lymph node pathology and histological changes.

Results

a. 8 weeks after the VX2 orthotopic tissue was implanted, axillary enlarged lymph nodes could be touched on 24 out of the 30 rabbits that were used to establish the model. The success rate was 80%. The pathological results showed that the enlarged lymph nodes were metastatic ones.

b. The images of two groups of lymph nodes routine scan by MRI: the lymph nodes in the contrast group were nearly circular ones with a smooth border, and lymph nodes of the experimental group were irregularly shaped ones with a coarse border. The enhancement scan showed: lymph nodes of the contrast groups appeared as even and obvious enhancement with a peak at 60 min, furthermore, the enhancement lasted for a long time and the enhancement rate was still more than 100%; lymph nodes of the experimental groups appeared as irregular or eccentric enhancement with a lower peak than that of the contrast groups, the enhancement lasted for a shorter time and after the peak at 60 min, the strength reduced. The standardization (SI) of two groups of lymph nodes after HD120-GD-DTPA enhancement was 2.76 ± 0.39 and 2.56 ± 0.25 respectively, and the corresponding average enhancement rate (En%) were 141.5% and 128% respectively, which means that the SI and En% of metastatic lymph nodes of the tumour were lower than that of normal lymph nodes and the difference between the two groups was statistically significant ($P<0.05$).

c. After 12 weeks of seed implantation, 9 out of 10 rabbits had shorter lymph nodes that could be touched, which means that the treatment was effective; the treatment of the last one was unsuccessful but the volume growth was obviously slower than that of the contrast group; the length was 3.28 cm when 8 weeks and particle aggregation could be shown by pathological anatomy. Rabbits of the contrast group all died and the enlarged lymph nodes started rupturing. The autopsy shows 5 cases of lung metastasis, 1 case of lung with liver metastasis, 2 cases of liver metastasis, and 1 case of bone metastasis. The difference in diameter between the two groups was statistically significant ($p<0.01$) from the 3rd week. Among all the rabbits, one relapsed and had a second excision and hasn't relapsed after that, complications such as upper limb edema and subcutaneous edema were observed in none of the other rabbits.

d. Pathologic characteristics of the lymph nodes: Gross observation showed an uneven surface, irregular shape, disappearance of hilar lymph nodes, plenty of macrocapillaries on the surface; multiple tumor metastases with a fish-like appearance and unclear border could be observed from longitudinal views. Microscopic appearance: the normal structure was destroyed; the amount of lymphocytes was reduced and lymphocytes were replaced by numerous bigger oval tumour cells; the nuclei of tumour cells with marked pleomorphism and obviously enlarged ratio of nucleus to cytoplasm could be seen.

Discussion

With the gradual development of the disease, breast cancer has become the leading cause of cancer mortality in women under the age of 45 [1], and the median survival rate of metastasis is not more than 24 months, so its early diagnosis and treatment are crucial [2]. Lymph node metastasis is the most common route of metastasis, so axillary lymph node status and its treatment are important. Some experiments on metastatic lymph nodes of breast cancer are not suitable for study in human beings, so the establishment of relevant animal models is an important part of the study of metastatic lymph nodes of breast cancer, and reliable evaluation methods are also of great significance [3]. The VX2 tumour cell line originates from Shope virus-induced rabbit papilloma-derived squamous cell carcinoma, and its high survival rate makes them suitable candidates for in vivo inoculation [4]. This model has been used to establish rabbit tumour models in various sites including uterus, tongue, stomach, hypopharynx, breast, rectum, muscle, cheek, liver, and lung, and is prone to lymph node metastasis [5]. Transplantation breast cancer model is the most widely used, transplantation methods include cell suspension injection, tissue block suspension inoculation and tumour tissue block inoculation, domestic and foreign studies have confirmed that tissue block inoculation is prone to lymph node and multi-organ metastasis, the possible reason is that the tissue block is small, the tumour cells can obtain sufficient nutrition and oxygen supply, and the concentration of tumour cells can resist the immune barrier, delay the clearance process, and ensure the normal growth of tumour cells. In this study, the tumour tissue block inoculation method was used to establish a breast cancer metastatic lymph node model, and the tumour growth rate was higher, and the tumour grew faster and larger, which was more suitable for the establishment of experimental rabbits.

Conclusion

a. Using the tissue block embedding method to establish the model of rabbit breast cancer lymph node metastasis is simple, easy, and highly effective.

b. The biological behaviour of the breast cancer model established by VX2 transplanted tumour was similar to human breast cancer.

c. The enhancement degree of HD120-GD-DTPA was the highest at 60 min after injection.

d. The new lymphoid targeting contrast agent HD120-GD-DTPA can be used for lymph node imaging and identification of the benign or malignant nature of lymph nodes.

e. 125I can effectively inhibit the growth of metastatic lymph nodes, and it is expected to be used clinically.

References

- Giaquinto AN, Sung H, Miller KD, et al. Breast cancer statistics, 2022. *CA Cancer J Clin* 72(6): 524-541.
- Wang W, Shen HY, Zhang Q (2023) The Dynamic Observation of Sentinel Lymph Nodes by Percutaneous Contrast Enhanced Ultrasound in Rabbit Model of Lymphatic Metastasis of Breast cancer. *Chinese Journal of Ultrasound in Medicine* 39(03): 333-336.



3. Yan LJ, Zhou J, Tian S (2022) Establishment of Rabbit Metastatic Lymph Node Model of Breast Cancer and Evaluation of Common Ultrasound Techniques. *Journal of Medical Research* 51(06): 93-97.
4. Yi HM, Cai BH, Ai X, Li K, Zhang W (2019) Establishment of rabbit liver VX2tumor model using percutaneous puncture inoculation of tumor fragment guided and evaluated by ultrasonography. *Curr Med Sci* 39(5): 820-824.
5. Gregor A, Fujino K, Bernards N, Kinoshita T, Mootoka Y, et al., (2019) Rabbit VX2 lung tumor models can form early nodal metastases. *World J Surg Oncol* 17(1): 231-237.

