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ORIGINAL ARTICLE

Experimental study on the relationship between traumatic stress and tumor growth, proliferation, and metastasis*

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Abstract

Objective This study aimed to investigate the relationship between traumatic stress and tumor growth, proliferation, and metastasis.

Methods A scalding method was used as an injurious factor to induce traumatic stress in Wistar rats. The rats were randomly divided into three groups—the control group, mild-scald group, and severe-scald group, with 14 rats in each group. Wistar rats were used to subculture the Walker-256 cell line for the generation of tumor ascites. Tumor cells from the ascites were cultured and used to establish a rat subcutaneous xenograft model. After 7 days, the mild-burn group and the severe-burn group were subjected burns to 10% and 15% of their backs, respectively. Blood was taken from the tail vein of rats at different times to detect changes in blood cortisol, IL-1β, and TNF-α levels. Pathological specimens were collected 14 days later, and immunohistochemistry was performed to examine vascular endothelial growth factor (VEGF), proliferating cell nuclear antigen (PCNA), E-cadherin, and vimentin.

Results Cortisol, IL-1β and TNF-α levels were significantly higher in the scalding groups than in the control group. Tumor examination was performed after 14 days. The changes in tumor size showed that the tumor volume in the control group (0.593 ± 0.195 cm^3) and the mild-scald group (0.782 ± 0.344 cm^3) were not significantly different. However, the tumor volume was significantly larger in the severe-burn group (1.806 ± 0.838 cm^3) than in the control and the mild-burn groups($P < 0.05$). Tumor tissue immunohistochemistry showed that the percentage of cells expressing PCNA in the control group, mild-scald group, and severe-scald group was 57.1%, 71.4% and 85.7%, respectively, and the differences among the groups were statistically significant. The number of VEGF-positive cells in the mild- and severe-scald groups was significantly higher than those of the control group ($P < 0.05$). The number of E-cadherin-positive cells in the tumor tissues was significantly lower in the severe-scald group than that in the control and mild-scald groups. Vimentin showed the opposite trend in the tumor tissue, and the differences were statistically significant ($P < 0.05$).

Conclusion Different degrees of a traumatic response in tissues caused by scalding can cause a corresponding stress response in the body. The release of inflammatory mediators; increase in VEGF, PCNA and vimentin in the tumor tissue; and decrease in E-cadherin lead to a change in tumor tissue growth and metastasis. Traumatic stress is associated with tumor growth, proliferation, and metastasis.

Key words: stress response; Wistar rats; tumor; scalding

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Surgery is the treatment of choice for cancer. However, the recurrence and metastasis of postoperative tumors is the main factor that affects the efficacy of treatment. Surgical traumatic stress interferes with the function of the immune system through the bidirectional regulation of the neuroendocrine system and the immune system,

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ultimately inducing and accelerating the development of the tumor and the surveillance of the tumor cells by the immune system [1]. However, the effect of the stress caused by different degrees of trauma on tumor growth and metastasis are unclear. In this study, a stress response induced by scalding rats was used as a model to study this problem.

Materials and methods

Materials

Forty-two male Wistar rats and 1 young Wistar rat were provided by the Experimental Animal Center of Beijing Academy of Military Medical Sciences. The average body weight of the rats was 120 g (120 ± 20) g. The young rats were weaned for 2 weeks and were used to generate ascites tumors to provide tumor cells. The Walker-256 cell line was provided by the PLA General Hospital.

Preparation and collection of tumor cells

The frozen tube containing the Walker-256 cell line was removed from the -80 °C liquid nitrogen freezer and thawed in a 37 °C water bath before washing with PBS. The supernatant was removed, and 2 mL of PBS was added. The cells were resuspended. One milliliter of cell suspension was injected into the abdominal cavity of the young rat. After 5 days, the ascites of the young rat was collected, washed with approximately 30 mL of PBS three times, and centrifuged to prepare concentrated tumor cells, which were used for further study.

Generation of a rat tumor model

The model was made by subcutaneously transplanting the tumor cells. Tumor cells were counted, and a suspension with a cell concentration of approximately 2×10^8/ mL was made with PBS. Injections with 0.3 mL of cell suspension, were targeted to the left groin of Wistar rats. After 5 days, tumor nodules with a diameter of approximately 0.3 cm were deemed to be successful.

Grouping and injury of the experimental animals

Days after inoculation, scalding was used as an injury method to induce systemic stress. Rats were divided into three groups—the control group, mild-scald group, and moderate-scald group, with 12 rats in each group. According to the Rubner formula, the body surface area of the rat S (m^2) = 0.0913 × body weight (kg) and the scald area = S × percentage of burned area (%). A rectangular template for the different areas was generated. The scalding model was executed as follows. In the mild-burn group, the rats were fixed on a wooden board, the skin of the back was immersed for 5 s in 100 ° C water; the scalded area was approximately 10% of the skin on the back. In the severe-scald group, the rats were fixed on wooden boards, and the skin protruding from the back was immersed in 100° C water; the scalded area was approximately 15% of the skin on the back. Immediately after the scalding, the animals were dried with dry gauze, and the back was coated with iodophor to prevent infection. According to the degree of the scalding, the rats were intraperitoneally injected with physiological saline solution. In the experimental control group, no injury or fluid replacement was performed, however, the rest of the treatments were the same as those in the experimental group.

Indicator detection

Serum cortisol, IL-1β, and TNFα levels were determined by ELISA at different times. Fourteen days after the systemic injury, the rats were sacrificed, and the tumor tissues were observed with light microscopy. Immunohistochemistry was used to detect the expression of proliferating cell nuclear antigen (PCNA), vascular endothelial growth factor (VEGF), E-cadherin, and vimentin in the tumor tissues.

Statistical analysis

SPSS 19.0 statistical software was used to analyze the measurement data, which are represented as mean ± standard, and each group was analyzed by one-way ANOVA. Multiple comparisons were performed with LSD or Dunnett T3 tests. The count data and rate were analyzed with a chi-square test.

Results

Tumor characteristics

Before the injury, the volume of the subcutaneous tumors in each group of rats increased without any spontaneous regression. There was no significant difference in the size of the tumors between the groups. After mild and moderate burns were generated, the tumor growth of the rats in the experimental groups was significantly different from that in the control group. Fourteen days after the injury, the rats were sacrificed, the tumor tissues of the rats were recovered, and the tumor sizes were measured. The results showed that the tumor volume of the control group was small and the morphology was regular. The tumor volume of the mild- and moderate-scald groups was significantly higher than that of the control group. The difference in tumor volume among the three groups was statistically significant ($P < 0.05$; Table 1).

Table 1 Change in the transplanted tumor volume in the rats of each group (cm^3) (n = 14)

Group	1 (d)	3 (d)	14 (d)
Control	0.044 ± 0.017	0.212 ± 0.098[a]	0.593 ± 0.195[a]
Mild-scald	0.058 ± 0.020	0.348 ± 0.186[ab]	0.782 ± 0.344[ab]
Severe-scald	0.059 ± 0.028	0.543 ± 0.639[ab]	1.806 ± 0.838[ab]

[a] Compared with the group on the first day; [b] compared with the control group (P < 0.05).

Changes in the various test indicators

There was a distinct inflammatory response caused by different types of trauma.

(1) Serum cortisol concentration: After the animal was injured, data were compared regarding changes over time and changes resulting from the degree of the injury. At different observation times, there was a significant difference in the serum cortisol levels between the same group of rats after injury. The rat serum cortisol began to rise 1 day after scalding, reached a peak after 3 days, and gradually decreased after 14 days; these differences were statistically significant. When comparing differences that resulted from the degree of the injury, the serum cortisol concentration in the control group (59.01 ± 8.51), mild-scald group (89.01 ± 12.26 μg/L), and moderate-scald group (129.01 ± 23.98 μg/mL) were significantly different one day after scalding (P < 0.05). The serum cortisol concentration in the severe-scald group (P < 0.05) was significantly higher than that in the control and mild-scald groups. This change persisted, with the lowest levels seen in the moderate-burn group on day 14 postinjury (Table 2).

(2) Serum IL-1β and TNF-α concentrations: The serum IL-1β and TNF-α levels based on the time and extent of the trauma also showed changes similar to those observed for cortisol. The differences among the cytokine levels in the groups at 1 day after scalding was statistically significant. The rat serum IL-1β and TNF-α levels began to rise 1 day after scalding and reached a peak after 3 days.

After 14 days, the degree of scalding was positively correlated with the increase in serum IL-1β and TNF-α levels, and the differences were statistically significant. (Tables 3 and 4).

Table 2 Changes in the plasma cortisol levels in the rats (μg/mL)(n = 14)

Group	1 (d)	3 (d)	14 (d)
Control	59.01 ± 8.51	61.08 ± 12.43[a]	60.07 ± 12.39[a]
Mild-scald	89.01 ± 12.26	100.12 ± 17.31[ab]	73.26 ± 15.62[ab]
Severe-scald	129.01 ± 23.98	141.01 ± 28.51[ab]	95.78 ± 15.44[ab]

[a] Compared with the group on the first day; [b] compared with the control group (P < 0.05).

Table 3 Changes in the plasma IL-1 β levels in the rats (n = 14)

Group	1 (d)	3 (d)	14 (d)
Control	5.8 ± 1.5	6.1 ± 1.9[a]	5.9 ± 1.7[a]
Mild-scald	91.8 ± 10.3	145.8 ± 17.5[ab]	40.2 ± 9.3[ab]
Severe-scald	156.2 ± 21.9	205.3 ± 26.4[ab]	55.78 ± 10.1[ab]

[a] Compared with the group on the first day; [b] compared with the control group (P < 0.05).

Table 4 Changes in the plasma TNF-α levels in the scalded rats (n = 14)

Group	1 (d)	3 (d)	14 (d)
Control	6.3 ± 1.7	6.5 ± 1.9[a]	6.5 ± 1.7[a]
Mild-scald	43.5 ± 7.5	65.0 ± 14.2[ab]	31.5 ± 9.7[ab]
Severe-scald	86.2 ± 14.9	124.1 ± 19.8[ab]	53.8 ± 12.3[ab]

[a] Compared with the group on the first day; [b] compared with the control group (P < 0.05).

Immunohistochemical expression of PCNA, VEGF, E-cadherin, and vimentin in rat tumor tissues

The expression of PCNA in the mild- and moderate-scald groups was higher than that in the control group. PCNA expression in the control group was noted in 50.0% of the tissue. However, PCNA expression in the mild-burn group was observed in 71.4% of the tissue, and its expression in the severe-burn group was noted in 85.7% of the tissue. These differences were statistically significant. The VEGF immunohistochemical analysis of the rat tumor tissue showed that the VEGF expression patterns were similar to those of PCNA. The positive proportion of E-cadherin in the tissue gradually decreased with the severity of scalding. In contrast, vimentin positivity gradually increased with the severity of the scalding (Table 5).

Discussion

While the biological behavior of tumors has received increasing attention, research on the relationship between the tumor and the whole organism has also continued to progress. Several studies have shown that tissue microenvironmental changes are closely related to tumor growth. Surgery has a significant effect on the

Table 5 Immunohistochemical expression of PCNA ,VEGF,E-cadherin and Vimentin in tumor tissue (n = 14)

Group	PCNA	VEGF	E-cadherin	Vimentin
Control	57.1%	35.7%	75.7%	14.3%
Mild-scald	71.4%[a]	64.3%[a]	68.3%[a]	21.4%[a]
Severe-scald	85.7%[a]	85.7%[a]	28.7%[a]	51.4%[a]

[a] Compared with the control group (P < 0.05).

recurrence and metastasis of some malignant tumors. The surgical removal of tumor lesions causes local changes at the surgical site and systemic changes in the body [1–2]. When the surgical trauma is greater, the traumatic stress response increases, including neuroendocrine reactions, cytokine changes, metabolic changes, and other possible biological responses [3-6]. The damage caused by different degrees of burns can lead to different degrees of systemic reactions. In this case, the hypothalamus, pituitary, and adrenal axes are excited, and corticosteroids are released in large quantities to maintain the stability of the body environment [7–8]. The results of this experiment also suggest that the amount of cortisol that is released differs based on the degrees of the burn; the level of corticosteroids released increases with the increasing burn severity.

The traumatic response of the body can further stimulate the systemic inflammatory response because of the stress experienced. Studies have shown that changes in inflammatory mediators, especially the release of large amounts of inflammatory mediators, can promote tumor progression by altering the tumor microenvironment [9]. An example of this is a massive release of TNF-α, which can eventually lead to the disruption of the body's homeostasis, changes in the tumor microenvironment, and the promotion of tumor growth. Studies have found that surgical trauma can cause sympathetic nervous system excitability throughout the neuroendocrine system, releasing catecholamines, which directly inhibit the function of immune cells (NK cells) and promote cytokine secretion by Th cells that indirectly inhibit cellular immune function, thereby promoting tumor cell growth and metastasis [10].

Another study has found that TNF can promote the expression of vascular endothelial cell surface adhesion molecules, thereby promoting the formation of tumor neovascularization [11]. Systemic blood redistribution during traumatic stress, including small blood vessel contraction, and paralysis can cause local tissue ischemia and hypoxia. The blood supply of tumor tissues can also be affected. Insufficient blood supply or ischemia-reperfusion injury occurs in tumor tissues, while the hypoxic environment can upregulate HIF-1 and regulate the expression of VEGF, increasing VEGF levels *in vivo*. VEGF can also accelerate the growth of solid tumors while promoting tissue regeneration [11–12]. As an important protein in cell proliferation, proliferating cell nuclear antigen (PCNA), also known as cyclin, also undergoes significant changes during the systemic inflammatory response. The expression of PCNA is closely related to the proliferation of cells. The detection of the number of PCNA-positive cells in tumor tissues can reflect the proliferation activity of tumor tissues and is a good marker for evaluating the state of cell proliferation. PCNA is currently widely used in the diagnosis and treatment of tumors and during prognostic evaluation [13]. In this study, the expression of PCNA in the experimental groups was higher than that in the control group ($P < 0.05$). The expression of PCNA in the severe-burn group was higher than that in the mild-burn group ($P < 0.05$), indicating that traumatic stress can enhance the expression of PCNA in tumor cells and promote tumor proliferation. E-cadherin maintains cell morphology, cell movement, and adhesion. Vimentin maintains cell shape, cytoplasmic integrity, and cytoskeletal stability. Decreased expression of E-cadherin changes the cytoskeleton, degrades the basement membrane, enhances activity, and causes high tumor invasiveness [12]. High expression of vimentin causes metastasis of tumor cells and can be used to evaluate the risk of metastasis. This study found that the expression of E-cadherin in tumor tissues gradually decreased with the increase in degree of scalding, but the expression of vimentin gradually increased as the degree of scalding increased. As the degree of scalding worsens, the stress response in rats increases, resulting in enhanced tumor invasion.

The study results indicate that different degrees of trauma lead to different degrees of the traumatic stress response and the resulting systemic inflammatory response accelerates tumor growth, through factors such as cell proliferation and vascular endothelial cytokines, and promotes changes to tumor cell morphology, movement, and activity. We speculate that with the increased stress response and the release of inflammatory factors, changes can occur in the tumor microenvironment and then activate the epithelial-mesenchymal transition of tumor cells, enhancing the movement capacity of tumor cells, which it turn may cause tumor cells to invade and metastasize. Tumor recurrence and metastasis are critical factors affecting survival after surgery. Thus, these issues deserve further study.

Conflicts of interest

The authors indicate no potential conflicts of interest.

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