Method Article

A method to establish a chronic restraint stress mouse model with colorectal cancer xenografts

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\textbf{A B S T R A C T}

Colorectal cancer (CRC) remains one of the most common clinical cancers of digestive tract. Recently, a large number of researches have shown that chronic stress can actively participate in the development of CRC. The proposed method successfully established the model of chronic stress mouse model with colorectal cancer.

- Chronic restraint stress (CRS) was used to establish chronic stress model.
- CRS was combined with a colorectal cancer xenografts model.
- Behavioural tests and tumour growth were used to evaluate model construction.

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Background

Colorectal cancer (CRC) is one of the most common clinical malignant cancers worldwide that is responsible for approximately 25% of cancer-related deaths every year [1,2]. Depression is a common psychological complication of CRC, and the presence of chronic stress has a major impact on depression [3,4]. Patients with CRC experience long and painful diagnosis and treatment processes, and continuous psychological stress often places these patients in a state of chronic stress [5,6]. Many clinical and epidemiological studies have confirmed that chronic stress increases the risk for CRC progression [7,8].

The method described in this research arms to further investigate the mechanisms of chronic stress and CRC.

Method details

Cell culture

Human colon cancer cell lines (HCT116 and LoVo) were acquired from the Cell Bank of the Institute of Cancer Research, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine. HCT116 cells were cultured and passaged conventionally at 37 °C in an atmosphere of 5% CO₂ and 95% air in RPMI-1640 (Gibco, USA) containing 10% foetal bovine serum (FBS).

Mice and animal care

Male athymic nude mice (NCr-nu), which were 6–7 weeks old and weighed 20–22 g, were purchased from Shanghai Sino-British SIPPR/BK. Mice were housed in a sterile laminar flow environment at the specific-pathogen-free (SPF) Experimental Animal Centre of Traditional Chinese Medicine at Shanghai University under a 12 h/12 h light/dark cycle at a temperature of 22 ± 2 °C and a relative humidity of 60 ± 5%, with access to a fresh food and water ad libitum. All experiments were in accordance with U.S. National Institutes of Health protocols in the Guide for the Care and Use of Laboratory Animals (8th edition).

Chronic restraint stress (CRS) model

Mice were bound to well-ventilated restraint 50 mL tubes without food and water from 8:00 AM to 16:00 PM for 14 consecutive days. The mice could not move freely or turn around but were not oversqueezed; this procedure induces chronic stress but not pain or injury. Mice in the control group were left in their usual environment and were undisturbed for 14 days (Fig. 1).

![Fig. 1. Schematic showing the timing of CRS and tumour injection.](image-url)
Fig. 2. Behavioral tests of CRS model. (A) The sucrose preference of the SPT in each group (n = 5). (B) The immobility time of the TST in each group (n = 5). **P < 0.01 compared with the control group.

Behavioral test

Sucrose preference test (SPT)
Before this test, all animals were simultaneously given two water bottles for 3 consecutive days, one containing water (200 mL) and the other containing a 1% sucrose solution (200 mL, Sigma-Aldrich). The placement of the 2 water bottles was reversed each day. Mice were deprived of water for 15 h before the test, which began at 8 AM. Each mouse was exposed to two identical weighed bottles (1% sucrose solution or water), and the bottles were then weighed after 8 h. Sucrose preference was calculated as the percentage of consumed sucrose solution relative to total liquid intake.

Tail suspension test (TST)
Mice were suspended 60 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for 6 min by an investigator blinded to group allocations. The immobility time was recorded during the last 4 min after 2 min of habituation. Mice were considered immobile only when they hung passively and were completely motionless. Any mice that climbed their tails were not considered in the experimental analysis.

CRC xenograft animal model
Mice were injected subcutaneously the resuspended HCT116 cells (2 × 10^6 cells in 100 μl). After subcutaneous xenograft, the tumour volume was measured using a vernier calliper every 3 days.

Statistical analysis
The survival analyses were carried out using GraphPad Prism (version 8.0), and Kaplan–Meier curves was used for the survival analyses.

Depressive behavior in the CRS mouse model with CRC xenografts
We confirmed the establishment of this mouse model by behavioural testing (Fig. 2). Compared with the control group (only injected tumour cells), the sucrose preference of the model group was lower (P < 0.01) and the tail suspension time was longer (P < 0.01).

Tumour growth and survival time of CRS model with CRC xenografts
Our results indicated that tumour volume was greater in the model group than in the control group. Moreover, analysis of overall survival (OS) indicated that the model group had the shorter OS (Fig. 3).
Fig. 3. Tumour growth and survival time of CRS model with CRC xenografts. (A) (a) Tumour growth curves (n = 10), (b) Overall survival curve. Survival rates were presented as Kaplan-Meier survival curves (n = 10). **P < 0.01 compared with the control group.

Declaration of Competing Interest

The authors have declared no conflict of interest.

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