Isolation and detection of label-retaining cells in a nasopharyngeal carcinoma cell line

Qing-Ping Jiang, Kai-Tai Yao

Abstract
Background and Objective: Detection of label-retaining cells (LRCs) has been a method to confirm existence of stem cells, and bromodeoxyuridine (BrdU) has commonly been used for labeling. In this study, to verify stem cells in nasopharyngeal carcinoma (NPC), LRCs were established and detected in NPC cell line 5-8F. Methods: The 5-8F cells were cultured with BrdU and inoculated subcutaneously into nude mice. By immunohistochemistry, immunocytochemistry, and immunofluorescence, BrdU was detected in 5-8F cells and xenograft tumors. Results: BrdU was strongly positive in cells on the 2nd and the 7th day after being added BrdU, while negative when cells were cultured without BrdU. However, only sporadic cells were positive on the 14th day after BrdU being washed-out, and these cells were thought to be LRCs. The average percentage of LRCs was (0.67 ± 0.32)%. After being cultured with BrdU for 48 h, 5-8F cells were inoculated into nude mice subcutaneously. After chasing 8 weeks, only sporadic LRCs were detected in xenograft tumors, with a proportion of (0.55 ± 0.36)% and these LRCs were located at cancer margin. Conclusion: The existence of LRCs in 5-8F cells indicates the existence of cancer stem cells in NPC.

Keywords: Label-retaining cells (LRCs), nasopharyngeal neoplasm, BrdU, stem cells

Materials and Methods

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incubated with 0.1 mol/L NaB₄O₇ for 8 min and washed with PBS, digested by 0.25% trypsin for 15 min and washed with PBS, then added with anti-BrdU antibody and incubated at 4°C overnight. After washing with PBS, HRP-goat anti-mouse IgG was added and slides were incubated for 30 min and washed with PBS. Finally, slices were stained with DAB and hematoxyline.

The scoring standards were as follows: according to cell staining, dark brown was scored 3 points, brown-yellow scored 2 points, light yellow scored 1 point and non-staining scored 0; according to the proportion of positive cells in a field of vision, >75% was scored 4 points, 51%–75% scored 3 points, 11%–50% scored 2 points, 1%–10% scored 1 point, and negative scored 0. Two scores were multiplied, 0–2 points was considered as negative, and ≥ 3 points was considered as positive expression.

Immunofluorescence of BrdU The protocol was as that above mentioned. Slides were incubated with BrdU antibody at 4°C overnight, then added with goat anti-mouse IgG-FITC and incubated for 30 min at room temperature and observed under fluorescence microscope. Evaluation criteria were as follows: clearly visible fluorescence was recored as +, very weak fluorescence as ±, and no fluorescence as -.

Results

Detection of LRCs cells in 5-8F cells

Immunocytochemistry and immunofluorescence showed that before BrdU was added, cells were negative; on the 2nd and 7th days after BrdU was added, nearly all cells were positive; while on the 14th day after discarding BrdU, only very few cells were positive, these cells were considered as LRCs. Randomly selected 10 high power fields to count, LRCs cells accounted for (0.67 ± 0.32)% of all cells (Figures 1 and 2).

Detection of LRCs in xenografts

After 8 weeks inoculation, with HE staining, cell morphology of xenografts were consistent and xenografts were poorly differentiated tumors with large areas of necrosis in the tumor nest. BrdU immunohistochemistry results showed that only a few cells were positive, which were LRCs. Randomly selected 10 high power fields to count, LRCs cells accounted for (0.55 ± 0.36)% of all cells (Figure 3).

Discussion

Compared with non-stem cells, the most prominent characteristic of stem cells is that few stem cells divide and the velocity of division is slow, known as slow-cycling cells. In 1981, Bickenbach et al. developed label-retaining method to identify stem cells by applying the characteristic of stem cells. They repeatedly injected tritium-labeled thymidine or thymidine analogs...
Incorporation into early all cellular DNA on the 48th hour, all 48th hour and the 7th day respectively, and found that BrdU immunohistochemistry and immunofluorescence staining on the we cultured 5-8F cells with BrdU for 7 days, applied using this method to detect LRCs in NPC. In the present study, these cells were considered to be LRCs cells. There is no report BrdU-positive on the 14th day after being washed out BrdU. In 10 high power fields, positive cells accounted for 0.67%, which were LRCs. The results are consistent with those in retinoblastoma report. Zhang et al. [10] has proved the existence of LRCs in mouse nasopharyngeal epithelia and NPC cell line by applying BrdU for in situ and xenograft labeling in adult stem cells of mice nasopharyngeal epithelium and NPC cancer stem cells. We cultured 5-8F cells for 48 h with BrdU and injected them into the bilateral armpit of nude mice for tumor formation. After 8 weeks, tumors were extracted and detected. The results showed that there were sporadic LRCs (0.55%) in xenografts, which were similar to that Zhang et al. [10] reported. However, whether these cells have the characteristics of stem cells needs to be further confirmed from the aspects of high tumorigenicity, sphere formation and co-expression of BrdU with other stem cell surface markers.

In summary, our study confirms the existence of NPC stem cells in view of LRCs.

References