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Labeling and tracking of mesenchymal stromal cells with EdU

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Background aims

The thymidine analog bromodeoxyuridine (5-bromo-2-deoxyuridine; BrdU) has been used widely to label cells in culture and in tissue. The labeled cells can also be tracked when transplanted into a suitable host. In the present study we tested a new thymidine analog, 5-ethynyl-2-deoxyuridine (EdU), for labeling and tracking of mesenchymal stromal cells (MSC), specifically adipose tissue-derived stem cells (ADSC).

Methods

Labeling of ADSC was examined for the dosage effect of EdU and stability of label by Alexa-594 staining followed by fluorescence microscopy. Labeling of various organs/tissues was done by intraperitoneal injection of EdU and examined by histology and fluorescence microscopy. Tracking of ADSC was done by intratissue or intravenous transplantation of EdU-labeled ADSC into various tissues and examined by histology and fluorescence microscopy.

Results

EdU was incorporated specifically into the nucleus in approximately 50% of ADSC and the percentage of cells that remained fully labeled declined with time. Peritoneal injection of EdU resulted in the appearance of EdU-positive cells in most organs and tissues. In the intestine, EdU-positive cells were found in both the epithelium and connective tissues 7 h after injection. Long-term (2–6 week) follow-ups found EdU-positive cells only in the connective tissue. Tracking of ADSC was successful in tissues 10 weeks after intratissue or intravenous transplantation.

Conclusions

Cell labeling with EdU in culture or living animals can be performed easily. The detection of EdU label requires no harsh treatment or immunologic reaction, as detection of BrdU label does. EdU can be used for long-term tracking of ADSC.

Keywords

adipose tissue-derived stem cells, cell labeling, cell tracking, EdU, mesenchymal stromal cells.

Introduction

Bromodeoxyuridine (5-bromo-2-deoxyuridine; BrdU) is a synthetic nucleoside that can be incorporated into the newly synthesized DNA of replicating cells. The BrdU-containing cells can be detected subsequently by immunochemistry using a BrdU-specific antibody [1,2]. In earlier studies this BrdU-labeling method was used mainly for analyzing the cell cycle in cultured cells [3] and visualizing proliferating cells in the central nervous system [4]. In more recent studies, it has been used to identify stem cells, which are believed to divide slowly or to segregate chromosomes asymmetrically, with retention of BrdU in the slowly dividing stem cells or in the daughter (non-differentiating) stem cells but not in the differentiated daughter cells [5–8]. In addition, BrdU-labeling has been used to track stem or non-stem cells that are labeled *in vitro* and subsequently transplanted *in vivo* [9,10]. In the case of stem cells, such tracking allows determination of whether the transplanted stem cells have differentiated into a particular cell type [10,11].

However, despite its extensive usage in conventional cell biology and recent stem cell studies, the immunochemical detection of BrdU-labeled cells can be problematic because of the need to use strong DNA denaturing conditions, such as strong acids and heating, to expose the epitope so that it can bind to the antibody. This harsh treatment can distort the cell and tissue structure and can cause the loss of antigenicity of

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cellular proteins, preventing the immunochemical detection (co-localization) by their specific antibodies. Thus, in an effort to overcome these problems, Salic and Mitchison [12] recently introduced an alternative thymidine analog, 5-ethynyl-2-deoxyuridine (EdU). The terminal alkyne group of EdU allows chemical detection using a fluorescent azide that covalently binds to the alkyne group. This detection method is fast and specific and does not require DNA denaturation. Its application for visualizing proliferating cells in the central nervous system has recently been demonstrated [13]. In the present study we show that it can also be used to label and track stem cells, in this case mesenchymal stromal cells (MSC) derived from adipose tissue (adipose tissue-derived stem cells; ADSC) [14].

Methods

Animals

All animal experiments in this study were approved by the Institutional Animal Care and Use Committee, University of California, San Francisco. All animals were Sprague–Dawley rats purchased from Charles River Laboratories (Wilmington, MA, USA). For *in vivo* EdU-labeling, a total of 40 pups was used. These pups were given birth by rats that were induced to develop urinary incontinence (UI) as described previously [15,16]. Briefly, immediately after delivery, the rats were subjected to vaginal balloon inflation to simulate birth trauma. One week later, the rats underwent ovariectomy to simulate menopause. Fat pads of the excised ovaries were used for ADSC isolation. For tracking transplanted cells in a high-fat diet-induced UI model, a total of 20 3-month-old male rats was used. The experimental procedure for the establishment of the hyperlipidemic model has been described previously [17]. Briefly, rats were fed with a diet consisting of 2% cholesterol and 10% lard. Five months later, after body weight and blood lipid measurement, these rats were used for ADSC isolation and transplantation.

ADSC isolation and culture

ADSC were isolated from the above-harvested adipose tissue using a modified version of our previously published protocol [18]. Briefly, within 4 h of harvest, the tissue was rinsed with phosphate-buffered saline (PBS) containing 1% penicillin and streptomycin, minced into small pieces, and then incubated in a solution containing 0.075% collagenase type IA (Sigma-Aldrich, St Louis, MO, USA) for 1 h at 37°C with vigorous shaking. The top lipid layer was removed and the remaining liquid portion centrifuged at 220 *g* for 10 min at room temperature. The pellet was treated with 160 mM NH_4Cl for 10 min to lyse red blood cells. The remaining cells were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), filtered through a 40-μm cell strainer (BD Biosciences, Bedford, MA, USA) and plated at a density of 1×10^6 cells in a 10-cm dish. After reaching 80% confluence, the cells were trypsinized and reseeded at 25% confluence in successive passages. Cells between the second and fifth passages were used for EdU-labeling and tracking.

EdU-labeling of ADSC

For the EdU dosage study, 50 000 cells were seeded onto a coverslip in each well of a six-well plate in DMEM supplemented with 10% FBS, 1% non-essential amino acid, 10 000 U/mL penicillin, 10 000 mcg/mL streptomycin SO_4, 0.025 mg/mL fungizone and 110 mg/mL sodium pyruvate. Twenty-four hours later, EdU (Invitrogen, Carlsbad, CA, USA) was added to the medium in concentrations of 0, 10, 20 and 50 μM. Another 24 h later, cells were fixed for EdU staining (see below). For the time–course study (retention of label), 300 000 cells were seeded into a 10-cm dish in the same medium as above, and 24 h later EdU was added to the medium at 10 μM. After a further 24 h, cells were washed three times with PBS followed by the addition of culture medium and resumption of incubation. At days 1, 4, 7, 14 and 21, the cells were trypsinized and reseeded onto coverslips as above. Six hours after seeding, the cells were fixed with methanol, washed twice with PBS, incubated in 3% bovine serum albumin (BSA) in PBS, and incubated in 0.5% Triton® X-100 in PBS for 20 min at room temperature. The cells were then incubated with freshly made Click-iT reaction cocktail, which contained Alexafluor 594 azide (Alexa-594, catalog number C10084; Invitrogen), for 30 min at room temperature without light. Cells were counterstained with Hoechst (nuclear staining) and mounted in standard mounting media. The stained cells were examined with a Nikon Eclipse E600 fluorescence microscope and photographed with a Retiga 1300 Q-imaging camera. For determination of the percentage of EdU-positive cells, different cell samples were photographed with the same camera settings for blue (nuclear staining) and red (EdU-positive) fluorescence. From the photographs of each cell sample, the ratio of red versus blue fluorescent cells, regardless of intensity (total 1000 blue fluorescent cells), was determined as the percentage of EdU-positive cells.