

Report

Imaging of Nucleolar Dynamics During the Cell Cycle of Cancer Cells in Live Mice

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Original manuscript submitted: 07/08/2007

Revised manuscript submitted: 08/10/2007

Manuscript accepted: 08/10/2007

Previously published online as a *Cell Cycle* E-publication:
<http://www.landesbioscience.com/journals/cc/article/4861>

KEY WORDS

dual-color cancer cells, imaging, nucleolus, in vivo

ABSTRACT

The synthesis and assembly of ribosomal subunits take place in the nucleolus. The nucleolus forms in the nucleus around the repeated ribosomal gene clusters and undergoes cyclic changes during the cell cycle. Although the nucleolus is easily visualized by light microscopy of cells in vitro, the nucleolus has not been imaged in cells in vivo. We report here development of a mouse model to visualize the nucleolus cycle of cancer cells in live mice. HT-1080 human fibrosarcoma cells were labeled in the nucleus with histone H2B-GFP and with retroviral RFP in the cytoplasm. The nucleolus was visualized by contrast to the fluorescence of GFP expressed in the nucleus. HT-1080 dual-color cells were seeded on the surface of a skin-flap of nude mice. The inside surface of the skin-flap was directly imaged with a laser scanning microscope 24 hours after seeding. The nucleoli of the cancer cells were clearly imaged in real-time. The appearance of the nucleoli changed dramatically during the cell cycle. During mitosis, the nucleolus disappeared. After mitosis, the nucleoli decreased in number and increased in size. The nucleolus appears to have a major role in cell cycle regulation. Nucleolar imaging could be used for more precise determination of cancer-cell position in the cell cycle in vivo.

INTRODUCTION

The synthesis and assembly of ribosomal subunits take place in the nucleolus.^{1,2} The processing of ribosomes is closely connected with cell growth and proliferation. The nucleolus appears to have a major role in cell cycle regulation by sequestering proteins which affect cell cycle progression.³ Although the nucleolus is obvious when viewed in the light microscope in vitro, visualization of nucleoli in vivo has not been accomplished. The nucleolus undergoes cyclic changes during the cell cycle. The efficacy of many anticancer drugs depends on the cell cycle of cancer cells.⁴⁻⁶ To visualize the nucleolus to precisely determine the position the cancer cells in the cell cycle in vivo could enhance the efficacy of chemotherapy. For this purpose, cancer cells can also be synchronized with hydroxyurea (for examples see refs. 7 and 8).

We report here development of an imageable mouse model to visualize the nucleolus of HT-1080 human fibrosarcoma cells in live mice.^{9,10} HT-1080 human fibrosarcoma cells were labeled in the nucleus with histone H2B-GFP and with retroviral RFP in the cytoplasm.¹¹ Nuclear GFP expression enables visualization of nuclear dynamics of HT-1080-GFP-RFP human fibrosarcoma cells. Simultaneous cytoplasmic RFP expression enables visualization of nuclear-cytoplasmic ratios as well as simultaneous cytoplasmic and nuclear shape changes. Thus, total cellular dynamics can be visualized in the living dual color cells in real-time.^{12,13}

Using the HT-1080 dual-color cells, the nucleolus was visualized by contrast to the fluorescence of the GFP expressed in the nucleus of the cancer cells. Correlations were made between the nucleolar cycle and cell cycle in order to more precisely determine the position of the cancer cells in their cell cycle in vivo.

MATERIALS AND METHODS

Establishment of dual-color cancer cells lines. To establish dual-color HT-1080 human fibrosarcoma cells, the cells were transfected with retroviral RFP and histone H2B tagged with H2B-GFP as previously described.⁹⁻¹⁵ In brief, the *Hind* III/NotI fragment from pDsRed2 (Clontech Laboratories, Inc., Palo Alto, CA) was inserted

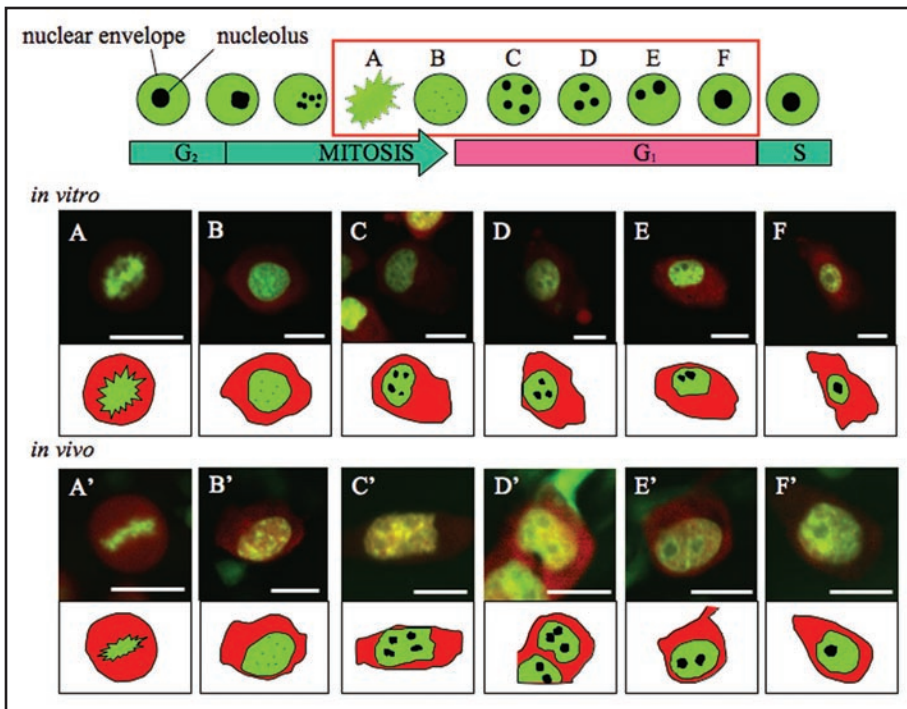


Figure 1. Real-time imaging of nucleolar dynamics during the cell cycle *in vitro* and *in vivo*. When the cells were entering mitosis, the chromatin was condensed (Fig. 1A and A') such that the nucleolus could not be visualized. After mitosis, the nuclei became rounded (Fig. 1B and B'). At this time, the nucleoli were not yet visible. In early G_1 , four nucleoli were visualized in each nucleus (Fig. 1C and C'). The nucleoli were light in contrast to the GFP expressed in the nucleus and were still small. As the cell cycle advanced, the nucleoli decreased in number and increased in size (Fig. 1D, D', F and F'). Bar, 20 μm .

into the Hind III/Not I site of pLNCX2, which also contains the neomycin resistance gene (*neo*[®]) (Clontech). For vector production, PT67, an NIH3T3-derived packaging cell line (Clontech) was incubated with a precipitated mixture of LipofectAMINE reagent (Life Technologies, Inc., Grand Island, NY) and saturating amounts of pLNCX2-DsRed2 plasmid for 18 h. For selection of a clone producing high amounts of a RFP retroviral vector (PT67-DsRed2), the cells were cultured in the presence of 200–1000 $\mu\text{g}/\text{ml}$ G418 (Life Technologies) for seven days.

The histone H2B-GFP fusion gene was inserted at the *Hind* III/*Cal* I site of the pLHCX (Clontech). To establish a packaging cell clone producing high amounts of histone H2B-GFP retroviral vector, the pLHCX histone H2B-GFP containing the hygromycin (HYG)-resistance gene (*hyg*[®]) plasmid was transfected in PT67 cells using the same methods described above for PT67-DsRed2. The transfected cells were cultured in the presence of 200–400 $\mu\text{g}/\text{ml}$ HYG.

For RFP and H2B-GFP gene transduction of HT-1080 cells, clones of HT-1080 expressing RFP in the cytoplasm were initially established. The cells were incubated with retroviral supernatants of PT67-RFP cells for 72 h. Cells were cultured in selective medium, which contained 200 $\mu\text{g}/\text{ml}$ G418. The level of G418 was increased stepwise up to 800 $\mu\text{g}/\text{ml}$. The cells were then incubated with retroviral supernatants of PT67 H2B-GFP cells. To select for double transformants, cells were incubated in selective medium with HYG for 72 h. The level of HYG was increased stepwise up to 400 $\mu\text{g}/\text{ml}$.

Real-time visualization of the nucleolus *in vitro* and *in vivo*. To observe the nucleolus of HT-1080 dual-color cells *in vitro*, the cells were cultured in RPMI supplemented with 10% heat-inactivated FBS in 10 cm dish (Corning, New York). When the cells were 50% confluent, the nucleoli were visualized directly with the IV100 laser scanning microscope (Olympus Corporation, Tokyo, Japan).

Mice. CD-1 nude mice were kept in a barrier facility under HEPA filtration.¹⁰ Mice were fed with autoclaved laboratory rodent diet (Tecklad LM-485, Western Research Products, Orange, CA). All animal studies were conducted in accordance with the principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory Animals under Assurance No. A3873-1.

CD-1 nude mice were anesthetized with a ketamine mixture (10 μl ketamine HCL, 7.6 μl xylazine, 2.4 μl acepromazine maleate and 10 μl H₂O) via s.c. injection. An arc-shaped incision was made in the abdominal skin and then subcutaneous connective tissue was separated to free the skin flap without injuring the epigastric cranialis artery and vein. The skin-flap was spread and fixed on a flat stand. A total of 100 μl medium containing 5×10^5 HT-1080 dual-color cells were seeded over the surface of the skin-flap. Twenty-four hours after seeding, the inside surface of the skin-flap was directly imaged with the IV100 laser scanning microscope. A total of three mice were analyzed.

RESULTS AND DISCUSSION

Real-time imaging of nucleolar dynamics during the cell cycle *in vitro* and *in vivo*. The non-fluorescent nucleoli of the HT-1080-GFP-RFP cells were visualized *in vitro* and *in vivo* by contrast to the fluorescence of GFP expressed in nucleus (Fig. 1). When the cells were in mitosis, the chromatin was highly condensed (Fig. 1A and A'), such that the nucleolus could not be visualized. After mitosis, the nucleoli gradually became visible and the nuclei became rounded (Fig. 1B and B'). In the early stage of the G_1 phase, four nucleoli were visualized in each nucleus (Fig. 1C and C'). The nucleoli were light in color and the sizes were still small. As the cell cycle advanced, the nucleoli decreased in number while increasing in size (Fig. 1D, D', F and F').

It has been reported that appearance of the nucleolus changes during the cell cycle.² We report here the correlation between nucleolar appearance and cell cycle position *in vivo* as well as *in vitro*. The nucleolus can thus serve as a marker for cell cycle position *in vivo*. Such information can be highly useful to determine the cell cycle dependence of cancer drug as well as many other applications.

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