The Use of Living Cancer Cells Expressing Green Fluorescent Protein in the Nucleus and Red Fluorescence Protein in the Cytoplasm for Real-time Confocal Imaging of Chromosome and Cytoplasmic Dynamics During Mitosis

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Abstract. *Background/Aim: A library of dual-color fluorescent cancer cells with green fluorescent protein (GFP), linked to histone H2B, expressed in the nucleus and red fluorescent protein (RFP) expressed in the cytoplasm was previously genetically engineered. The aim of the current study was to use the dual-color cancer cells to visualize chromosome and cytoplasmic dynamics during mitosis. Materials and Methods: Using an Olympus FV1000 confocal microscope, a library of dual-color cells from the major cancer types was cultured on plastic. The cells were imaged by confocal microscopy to demonstrate chromosome and cytoplasmic dynamics during mitosis. Results: Nuclear GFP expression enabled visualization of chromosomes behavior, whereas simultaneous cytoplasmic RFP expression enabled visualization of cytoplasmic behavior during mitosis. Thus, total cellular dynamics can be visualized at high resolution, including individual chromosomes in some cases, in living dual-color cells in real time. Conclusion: Dual-color cancer cells expressing H2B-GFP in the nucleus and RFP in the cytoplasm provide unique tools for visualizing subcellular nuclear and cytoplasm dynamics, including the behavior of individual chromosomes during mitosis. The dual-color cells can be used to evaluate chromosomal loss or gain in real time during treatment with a variety of agents or as the cells are selected for increased or decreased malignancy in culture or in vivo. The dual color cells will be a useful tool to discover and evaluate novel strategies for killing cancer cells.*

This paper is dedicate to the memory of A.R. Moossa, M.D.

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Until the advent of cloned fluorescent protein genes (1, 2), the study of nuclear-cytoplasmic dynamics was mainly carried out on fixed cells. Although vital dyes were of some use, visualization of chromosomes in living cells was difficult. In 1998, the laboratory of Geoff Wahl (3) created a fusion gene of histone H2B and green fluorescent protein (GFP), which made the study of nuclear dynamics in live cells practical (4). Subsequently, our laboratory created dual-color cells with H2B-GFP expressed in the nucleus and red fluorescent protein (RFP) expressed in the cytoplasm (4, 5), which enable real-time simultaneous imaging of nuclear and cytoplasmic behavior.

The dual-color cancer cells have been used to demonstrate novel cellular behavior including: nuclear and cytoplasmic destruction during infection by tumor-targeting *Salmonella typhimurium* (6); nuclear and cytoplasmic deformation by cancer cells trafficking in narrow capillaries (7, 8); clasmocytosis (destruction of the cytoplasm) of cancer cells *in vivo* in real time in the liver, circulation and other organs (9-11); nuclear-cytoplasmic behavior in real time of cells trafficking in blood vessels (12) and lymphatic vessels (13); nuclear and cytoplasmic separation during cancer cell death (10, 14); real-time apoptosis *in vitro* (4, 15) and *in vivo* (16-18); real-time mitosis *in vitro* (4) and *in vivo* (19, 20); macrophage ingestion and digestion of nuclei and cytoplasm (21, 22) and the role of nuclear rigidity in cancer-cell migration (7, 8, 23).

In the present report, a compilation of an atlas of dual-color cancer cells, expressing GFP in the nucleus and RFP in the cytoplasm, from the major cancer types is presented demonstrating chromosome and cytoplasmic dynamics during mitosis.

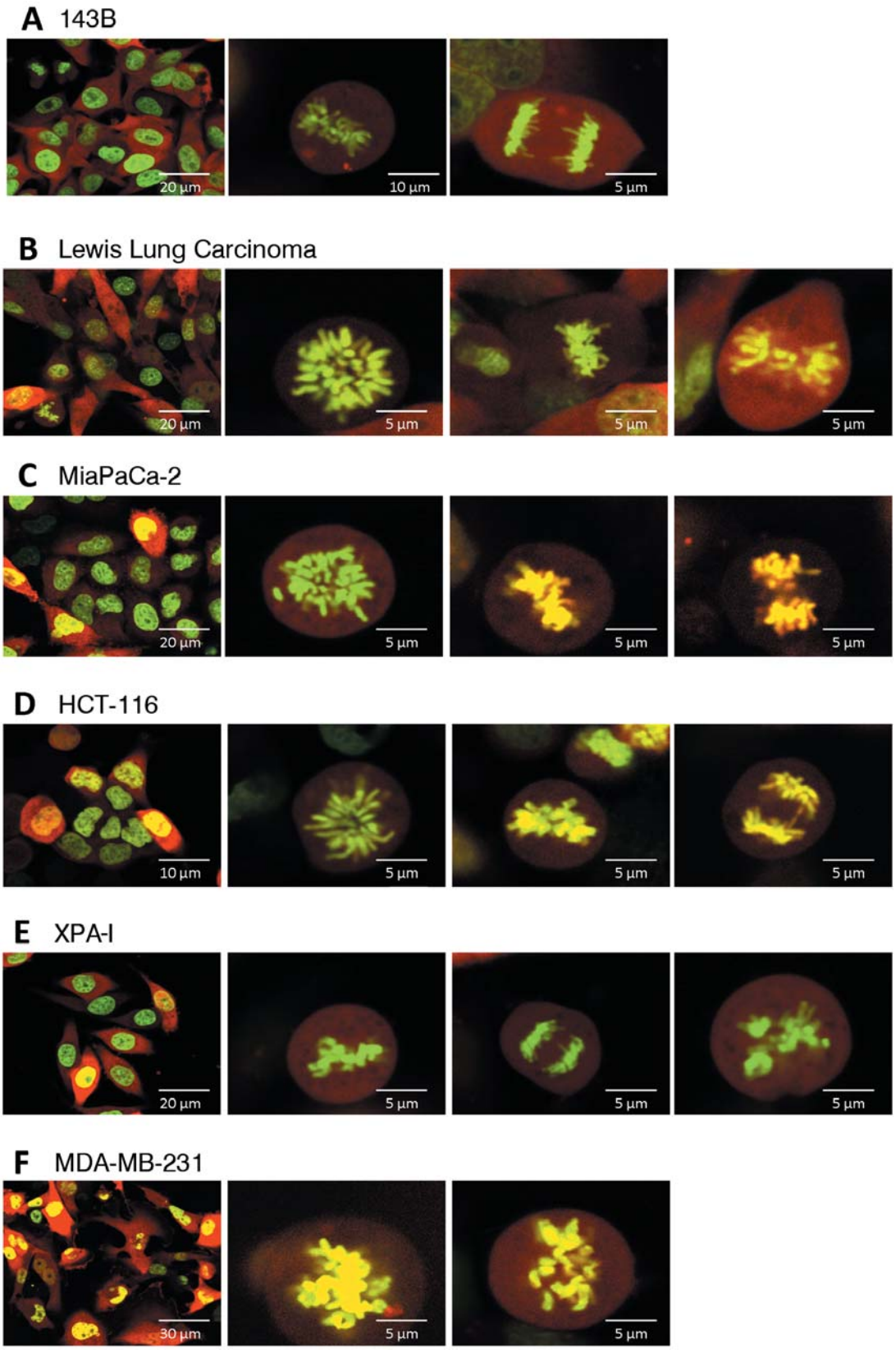

Figure 1. *Continued*

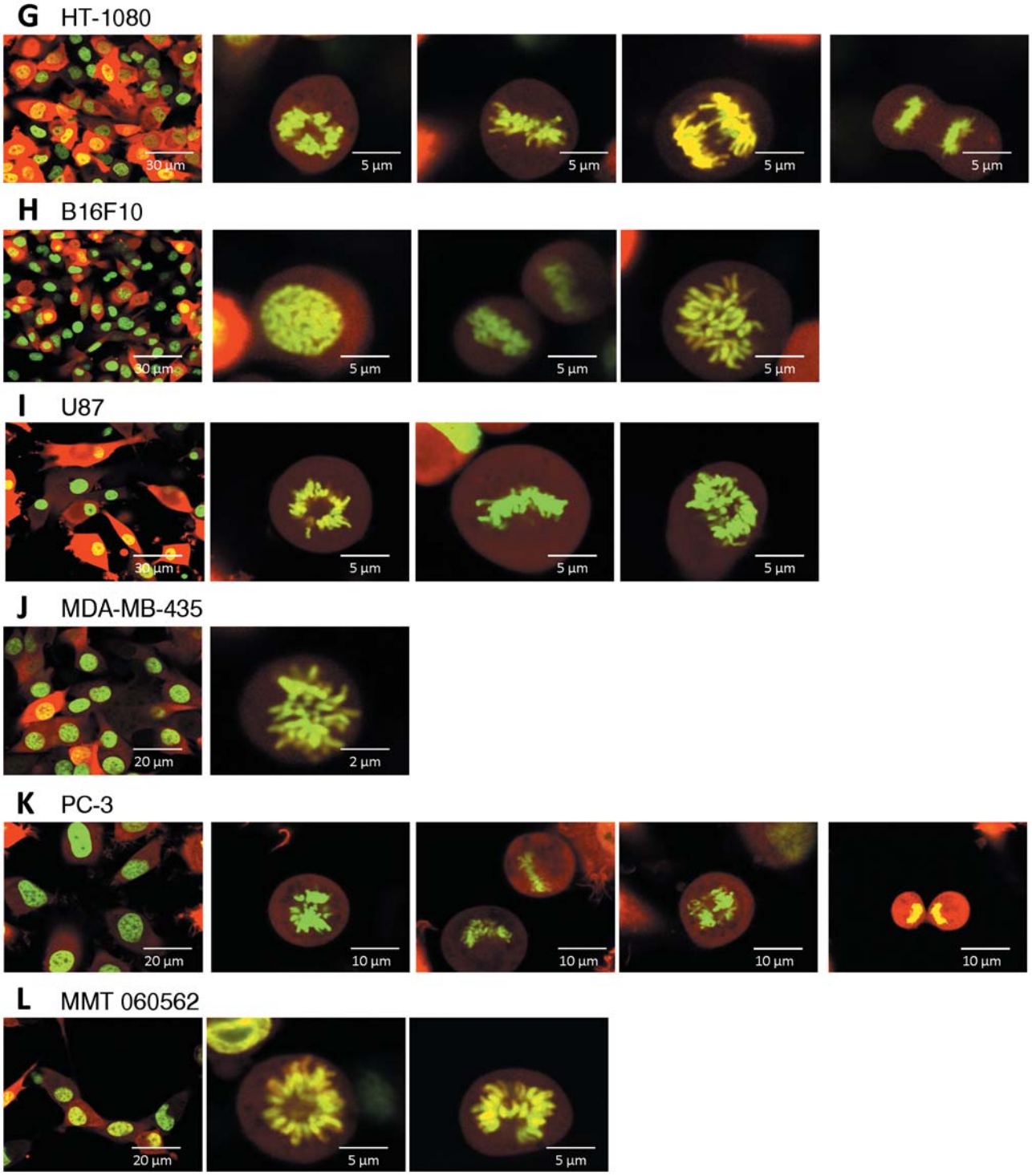

Figure 1. *Green fluorescent protein-red fluorescent protein (GFP-RFP)-expressing dual-color cancer cells in vitro. Cancer cells were initially transduced with RFP and the neomycin-resistance gene. The cells were subsequently transduced with histone H2B-GFP and the hygromycin-resistance gene. Double transformants were selected with G418 and hygromycin, and stable clones were established. See Materials and Methods for details. Images were captured under FV1000 confocal microscopy. A: 143B, human osteosarcoma; B: Lewis lung cancer, murine lung cancer; C: MiaPaCa-2, human pancreatic cancer; D: HCT-116, human colonic cancer; E: XPA-I, human pancreatic cancer; F: MDA-MB-231, human breast cancer; G: HT-1080, human fibrosarcoma; H: B16F10, murine melanoma; I: U87 MG human glioma; J: MDA-MB-435, human breast cancer; K: PC-3, human prostate cancer; L: MMT 060562, murine mammary tumor.*

Materials and Methods

Production of histone H2B-GFP vector. The histone H2B-GFP fusion gene (3) was inserted at the HindIII/ClaI site of the pLHCX (Clontech Laboratories, Inc.Palo Alto, CA, USA) that has the hygromycin-resistance gene. To establish a packaging cell clone producing high amounts of histone H2B-GFP retroviral vector, the pLHCX histone H2B-GFP plasmid was transfected in PT67 cells (Clontech Laboratories, Inc.). The transfected cells were cultured in the presence of 200-400 μg/ml hygromicyn (Life Technologies, Inc., Grand Island, NY, USA) for 15 days to establish stable PT67 packaging cells producing the H2B-GFP gene vector (4, 5).

Production of RFP retroviral vector. For RFP retrovirus production, the Hind III/NotI fragment from DsRed2 (Clontech Laboratories, Inc.), containing the full-length RFP cDNA, was inserted into the Hind III/NotI site of pLNCX2 (Clontech Laboratories, Inc.) that has the neomycin-resistance gene to establish the pLNCX2-DsRed2 plasmid. PT67 cells were cultured in DMEM (Irvine Scientific, Santa Ana, CA, USA) and 10% fetal bovine serum (FBS; Gemini Bio-products, Calabasas, CA, USA). PT67 cells at 70% confluence were incubated for 18 h with a precipitated mixture of Lipofectamine reagent (Life Technologies, Inc.), and saturating amounts of pLNCX2-DsRed2 plasmid. Fresh medium was replenished at this time. For production of high amounts of the DsRed2 gene vector, the PT67 cells were cultured in the presence of 200-1000 μg/ml G418 (Life Technologies, Inc.), increased in a step-wise manner, for seven days (4, 5, 24-26).

RFP and histone H2B-GFP gene transduction of cancer cells. To establish dual-color cells, clones of cancer cells expressing RFP in the cytoplasm were initially established by incubation with a 1:1 mixture of retroviral supernatants of PT67-RFP cells and RPMI-1640 (Mediatech, Inc., Herndon, VA, USA) containing 10% FBS for 72 h. Fresh medium was replenished at this time. Cells were harvested with trypsin/EDTA 72 h post-transduction and subcultured at a ratio of 1:15 into selective medium containing 200 μg/ml G418 which was increased stepwise up to 800 μg/ml (4, 5, 24, 25). The cancer cells expressing RFP were then incubated with a 1:1 mixture of retroviral supernatants of PT67 cells with the H2B-GFP vector. To select the double transformants, cells were then incubated with hygromycin which was increased stepwise up to 400 μg/ml (4, 5, 24-26).

Imaging. These cells were observed with an FV1000 confocal microscope (Olympus, Tokyo, Japan).

Results and Discussion

The following cell lines, all expressing H2R-GFP in the nucleus and RFP in the cytoplasm were imaged during mitosis by confocal microscopy. The features of the representative cell lines are listed below:

143B, Human osteosarcoma. The cells have bright GFP-expressing nuclei and RFP-expressing cytoplasm. Metaphase and anaphase are very well visualized (Figure 1A).

Lewis lung cancer, murine lung cancer. The cells have bright GFP-expressing nuclei and bright RFP-expressing cytoplasm. The chromosomes are well separated at prophase and metaphase and anaphase (Figure 1B).

MiaPaCa-2, Human pancreatic cancer. The cells have bright GFP-expressing nuclei and moderately bright RFP-expressing cytoplasm. The chromosomes are moderately separated at prophase and metaphase and anaphase and are well visualized (Figure 1C).

HCT-116, Human colon cancer. The cells have bright GFP-expressing nuclei and moderately bright RFP-expressing cytoplasm. The chromosomes are somewhat separated in prophase. Metaphase and anaphase are well visualized (Figure 1D).

XPA-I, Human pancreatic cancer. The cells have bright GFP-expressing nuclei and RFP-expressing cytoplasm. Metaphase and anaphase are well visualized, with some apparently aberrant mitosis taking place (Figure 1E).

MDA-MB-231, Human breast cancer. The cells have bright GFP-expressing nuclei and RFP-expressing cytoplasm. Prophase is well visualized. The chromosome are fairly well separated (Figure 1F).

HT1080, Human fibrosarcoma. The cells have bright GFP-expressing nuclei and RFP-expressing cytoplasm. The individual chromosomes are not well separated before metaphase, but prophase, metaphase, and anaphase are readily visualized (Figure 1G).

B16F10, Murine melanoma). The stages of mitosis are clearly visualized, with very bright GFP in the nucleus and RFP in the cytoplasm. The nuclear:cytoplasmic ratios become large before the cells enter metaphase. The chromosomes are well separated (Figure 1H).

U87 MG, Human glioma. The cells have bright GFP-expressing nuclei and RFP-expressing cytoplasm. Prophase, metaphase, and anaphase are well visualized. Individual chromosomes are fairly well separated (Figure 1I).

MDA-MB-435, Human breast cancer. The cells have very bright GFP-expressing nuclei and the cytoplasm has moderately bright RFP. The prophase chromosomes are fairly well separated (Figure 1J).

PC-3, Human prostate cancer. The cells have bright GFP-expressing nucleus and RFP-expressing cytoplasm. Prophase, metaphase, and anaphase are well visualized, but the chromosomes are not well separated (Figure 1K).

MMT 060562, Murine mammary tumor. The cells have very bright GFP-expressing nuclei and moderately bright RFP-

expressing cytoplasm, Metaphase, and anaphase are well visualized. The chromosomes are fairly well separated (Figure 1L).

The dual-color cancer cells and the Olympus FV1000 confocal microscope provide very high resolution of chromosome and cytoplasmic dynamics. The cell imaging techniques described here will enable future studies to further understand the mechanisms of cancer growth and progression at the subcellular level in particular at the chromosome level, *in vitro* and *in vivo* in real time.

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