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Long-Term Low-Intensity Live Cell Imaging of Therapeutically Treated Cells in Cultures

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Phase contrast, fluorescence and oblique incidence reflection imaging techniques have been used for the purpose of monitoring long-term cellular activity of therapeutically treated cancerous cells in cultures. Morphological changes in living cells that were induced by either irradiation or chemical agent were monitored over several days. Oblique incidence reflection (OIR) microscopy was used to obtain information on cell dynamics with minimal photodamage and sample degradation.

Time-lapse imaging and cell culturing techniques used in this study were as described in [1]. Both cell lines for which data are presented were monolayer-adherent type cultures of human origin. With the exception of a few complementary fluorescence frames, the OIR imaging method was used to gather time-lapse information from a targeted cell group.

Radiation is commonly and successfully used in the treatment of certain cancers, the dose being delivered in fractions or a sequence of incremental amounts. A preliminary assessment of the effects of irradiation was performed using cancer cells known to be responsive to radiation treatment, and a single dose fraction. A dose of 600cGy was delivered to histone H1-GFP labeled breast cancer cells (MCF-7) using the 6MV therapeutic x-ray beam from a Siemens ONCOR linear accelerator. Phase contrast images of randomly selected cell groups were taken each day for a period of 3 weeks post-irradiation. Figure 1 (top) shows representative images of both untreated control (a) and treated (b through d) cells at one-week intervals. Typically, major morphological changes were visible after 4 days; treated cells were many times larger than normal and contained multiple nuclei. Morphologically normal cell colonies began to appear after 1 week and eventually took over the culture after 3 weeks of incubation (untreated control became confluent after 1 week). Figure 1 e) through g) illustrate examples of still frames selected from a time-lapse imaging session performed 6 days post-irradiation. Sequences of oblique images provided clear visualisation of macromolecular streaming, structural movement, nucleoli dynamics, and cell death.

Induction of cellular differentiation is a possible treatment of cancers, and clinical trials are underway on several such inducers. Neuroblastoma cell lines have been used as a model to study cell differentiation [2,3]. To induce morphological changes typically seen in differentiation, untagged SK-N-SH neuroblastoma cells were incubated with 5mM sodium butyrate (a histone deacetylase inhibitor known to initiate differentiation in numerous cell lines). Time-lapse OIR imaging experiments of untreated control and treated cells were performed over several days. Figure 2 shows the typical morphology of untreated control (a), and cells treated for 12h (b), 24h (c) and 48h (d). Time-lapse sequences showed that subtle and transient morphological changes (such as narrow cell protrusions and cell elongation) appeared approximately 5 hours into treatment. Neuron-like morphological changes are clearly visible in Figure 2 c) and d).

References

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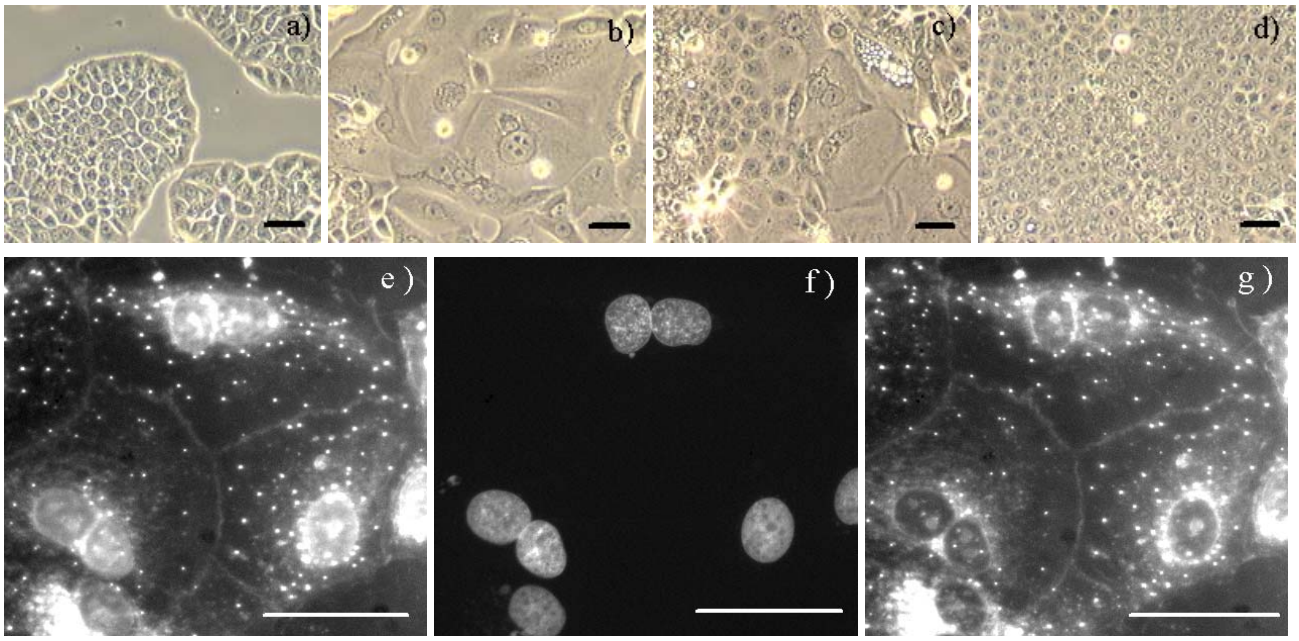

Fig. 1. Histone H1-GFP labeled breast cancer cells (MCF-7) irradiated with a single 600cGy dose. (Top) Phase contrast images of randomly selected cell groups: a) untreated control, b) to d) 7 days, 14 days and 21 days post-irradiation respectively. (Bottom) Examples of still frames selected and cropped from a time-lapse imaging sequence performed 6 days post-irradiation: e) combined fluorescence and OIR imaging, f) fluorescence imaging only, and g) OIR imaging only (macromolecules, cell boundaries and nucleoli are clearly visible). Scale = 50 microns.

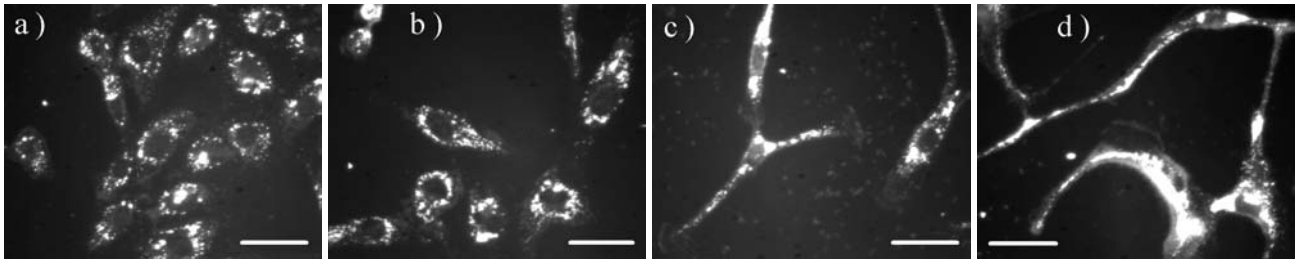

Fig. 2. OIR imaging of chemically treated untagged neuroblastoma cells (SK-N-SH): a) untreated control, and incubated with 5mM sodium butyrate for b) 12 hours, c) 24 hours and d) 48 hours. Scale = 50 microns.

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