Effects of the thymidine analogues EdU and BrdU on cell viability and cycle progression

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Background

A recently introduced method for determining S-phase synthesis (SPF) using the nucleoside analog EdU (5'ethynyl-2'-deoxyuridine) coupled with click chemistry has been proposed as a BrdU replacement. Long term exposure of cells to BrdU has been shown to alter cell cycle progress and distribution of cell cycle phases. Structurally similar to the natural nucleoside, both EdU and BrdU modification occurs in the same region of the pyrimidine ring. Although the alkyne is unreactive in biological systems, the effect on cell viability, DNA synthesis, and cell cycle checkpoints to long term exposure has not been explored so far.

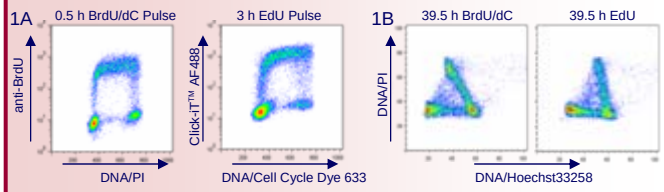

Fig. 1 Applicability of BrdU/dC and EdU for dynamic cell proliferation assessment in BT474. A: anti-BrdU (20 µM BrdU/ dC) vs. Click-iT EdU (20 µM EdU). B: BrdU/Hoechst Quenching vs. EdU/Hoechst Quenching. Cells were continuously exposed to 120 µM BrdU/60 µM dC or 100 µM EdU.

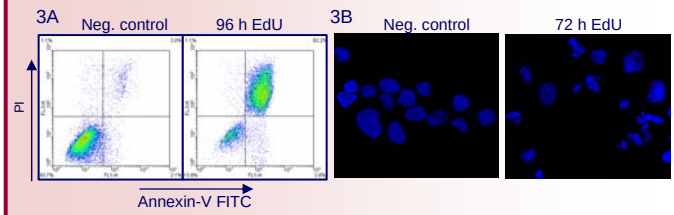

Fig. 3: Annexin-V FITC/PI (A) and DAPI (B) labeling of SK-BR-3 cells exposed to 20 µM EdU. EdU induces cell death by necrosis rather than by apoptosis.

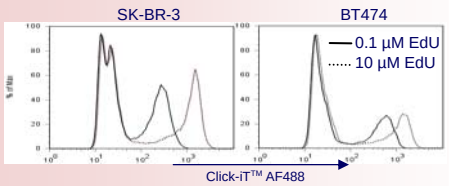

Fig. 4 Click labeled SK-BR-3 cells using Alexa Fluor® 488 azide pulse labeled with EdU for 3 hours compared to BT474 cells. EdU is efficiently incorporated into DNA of both cell lines even at the lowest concentrations.

Results

EdU treatment turnded out to be applicable for cell proliferation assessment applying Click-iT chemistry (Fig. 1A) and quenching of the DNA dye Hoechst33258 (Fig. 1B). However, cell viability of SK-BR-3 breast cancer cells was highly affected by long term exposure to EdU (Fig. 2A and 2B). Media addition of dC with EdU did not improve cell viability. In contrast, cell viability of BT474 was not affected by EdU treatment (Fig. 2C and 2D). EdU treatment causes cell death in SK-BR-3 (Fig. 3A and 3B) was due to greatly increased necrosis (Fig. 3A). Although in respect to cell viability both cell lines showed a different sensitivity to EdU: they incorporate EdU with almost the same efficiency even at low concentrations (Fig. 4). EdU caused G2/M phase cell cycle arrest can be observed in both SK-BR-3 and BT474 cells (Fig. 5).

Material and Methods

We examined the effect of long term (96 h) continuous exposure of EdU or BrdU/dC on SK-BR-3 and BT474 breast cancer cell lines. Cell cycle analysis with dead cell gating was measured on a BD LSRII (633 nm, 405 nm) using CellCycle 633-red and LIVE/DEAD® Fixable Violet cell stain. Apoptosis was evaluated with an Annexin-V FITC/ PI Assay. Incorporation of EdU into DNA was measured with Click-iT™ EdU Alexa Fluor® 488 Cell Proliferation Assay Kit. A BD FACSCalibur (488 nm; 635 nm) was used to analyze apoptosis and EdU-incorporation. DAPI stained cells were visualized with an AxioImager Z1 microscope.

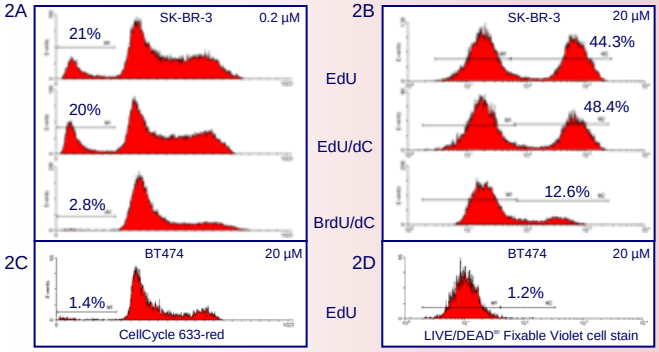

Fig. 2 Analysis of cytotoxicity measured by gating subG$_1$ (Fig. 2A and 2C) and dead cells (Fig. 2B and 2D) shows that 96-h long term exposure to EdU but not BrdU/dC increases the dead cell fraction in SK-BR-3 (Fig. 2A and 2B) but not in BT474 cells (Fig. 2C and 2D). DC does not rescue SK-BR-3 cells from EdU mediated cytotoxicity.

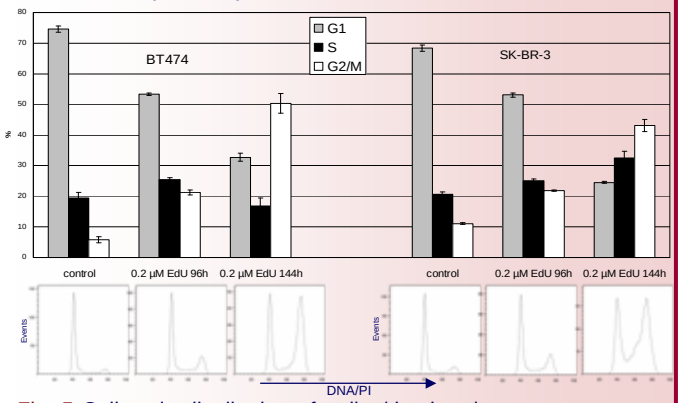

Fig. 5 Cell cycle distribution after live/dead gating upon treatment with EdU for 96 h and 144 h and respective DNA histograms are shown. EdU treatment causes G2/M arrest in SK-BR-3 and BT474.

Conclusions and Perspectives

Albeit thymidine/EdU replacement is highly appropriate for flow cytometric proliferation analysis the potential impact on cell viability needs to be evaluated. Analysis of cell proliferation using click chemistry can accurately be done using 1/200 of the standard BrdU concentration. Hence its anti-proliferative and/or necrosis inducing impact might be efficiently reduced by short time instead of continuous EdU labeling. Cytotoxicity of EdU on SK-BR-3 cells can not be attributed to increased EdU accumulation of EdU. Also BT474 cells efficiently incorporate EdU however they show significantly less necrosis. Further studies will elucidate the molecular mechanisms of EdU and BrdU mediated cell death and inhibited cell cycle progression.

References: [1] Diermeier S. et al. 2004. Cell Prolif. 37(2):195-206, [2] Salic A. et al. 2008. PNAS.105 (7):2415-2420. [3] Buck S.-B. 2008. Biotechniques. (in press)