Detecting real-time DNA synthesis using LigandTracer® White

By following the incorporation of ^{14}C-thymidine, LigandTracer White makes it possible to detect DNA synthesis in real-time. In the examples below we investigate how the nucleoside antimetabolite clofarabine and external radiation affect DNA synthesis rate.

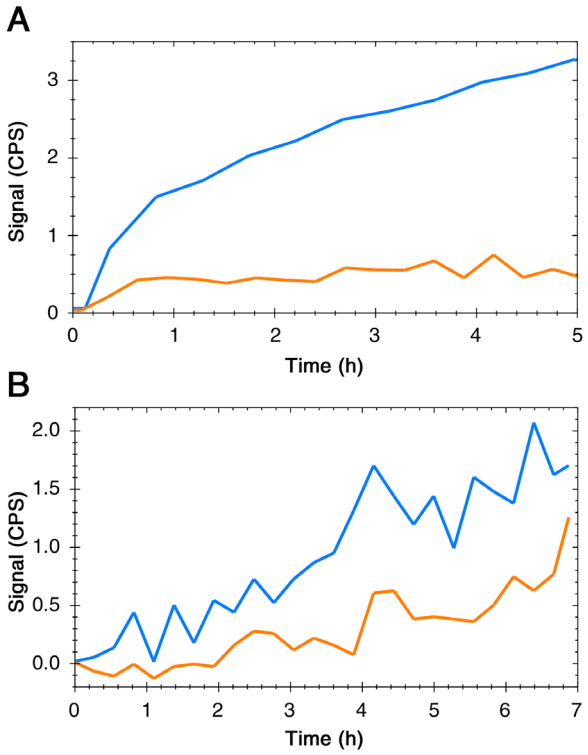
Experiment details

Effect of clofarabine on DNA synthesis

The uptake of ^{14}C-thymidine to HCT116 cells was monitored in a humidified incubator at 37 °C, using LigandTracer White (Fig. A, blue). In the presence of 10 µM clofarabine the ^{14}C-thymidine incorporation was greatly reduced (Fig. A, orange), showing the impact of the nucleoside analogue on the DNA synthesis rate.[1,3]

DNA synthesis rate after external radiation

A431 cells were seeded evenly in a circular cell dish. Prior to measurement, one cell free reference area was created by the use of a cell-scraper, and another area was shielded with lead. The dish was then irradiated with 10 Gy (662 keV, 1 Gy/min) and placed in LigandTracer White in a humidified incubator. The uptake of 46 kBq ^{14}C-thymidine to shielded cells (Fig. B, blue) and irradiated cells (Fig. B, orange) were simultaneously followed over time, showing a reduced DNA synthesis rate for the irradiated cells compared to the shielded ones. The same procedure was performed with 3 Gy, 6 Gy and 30 Gy, showing a DNA synthesis rate that was inversely proportional to radiation dosage. Also the recovery time increased with higher dosage (data not shown).[2]



Conclusions

DNA synthesis as monitored through ^{14}C-thymidine incorporation can be detected in real-time using LigandTracer White. The data can be used for the quantification of external irradiation effects on proliferation rate. Other applications include proliferation measurements following exposure to cytotoxic drugs or replication inhibitors, and onset of S-phase after G1 synchronization.

Reference and protocols

1. Wang X, et al. *Effect of clofarabine on apoptosis and DNA synthesis in human epithelial colon cancer cells.* Nucleosides Nucleotides Nucleic Acids. 2010. 29(4-6):414-418.
2. *We thank Prof. Bo Stenerlöw (Uppsala University, Sweden,) and Dr. Pavel Barta (Charles University, Prague, Czech Republic) for participating in this experiment.*
3. Protocol: Seeding Cells for LigandTracer®

Protocols can be downloaded at www.ridgeview.eu/download/