Abstract

Substantial public health concerns exist over the potential endocrine disrupting capabilities of a wide variety of untested or under-tested natural and industrial chemicals. It is clear that the development of accurate, high-throughput, and inexpensive testing regimens will be key to mitigating public concern. Here we report on the development of a novel screening assay for estrogenic activity that utilizes an autonomously bioluminescent human cell line to provide direct bioavailability data. To construct this cell line, estrogen-responsive human breast carcinoma cells (T-47D) were genetically engineered to express the full bacterial bioluminescence gene cassette (luxCDABEfrp), generating an autonomously bioluminescent cell line (T-47D/Lux) capable of maintaining bioluminescent output independent of substrate addition. Bioluminescence emitted from T-47D/Lux cells was correlated tightly (R² > 0.99) to the number of cells present in a population, permitting the use of light production dynamics as an indicator of cell proliferation. Additionally, the substrate-free nature of the lux system allowed for continuous, near real-time monitoring of the same cell population throughout exposure to the tested compounds. A significant change in bioluminescent production (p < 0.05) compared with unexposed control was observed 3 days after exposure to concentrations of 17β-estradiol (E₂) as low as 1 pM. The EC₅₀ for E₂ in this assay was determined to be approximately 10 pM. These results are similar to those obtained using a traditional cell proliferation assay, but offer the advantage that data acquisition can be performed in a fully automated fashion since the need for sample destruction or substrate addition is removed, making it an ideal candidate for high-throughput analysis.

The Bacterial Bioluminescence (lux) Reporter System

- Although widely used as a prokaryotic reporter system, the lux system has only recently been optimized for expression in mammalian cells
- In addition to the luciferase genes (luxAB), the luxCDABEfrp gene products generate and recycle the aldehyde and FMNH₂ co-substrates required for the light reaction
- Expression of all six genes allows for autonomous bioluminescent production without the addition of an expensive substrate

Continuous Monitoring of Estrogen-Induced Cell Proliferation

- Approximately 1 x 10⁴ constitutively bioluminescent T-47D/Lux cells were plated into each well in a tissue culture treated, black 24-well plate
- 17β-estradiol (E₂) was added at final concentrations ranging from 0 pM (control) to 100 nM in triplicate wells
- Bioluminescence was measured every 24 hours for 6 days (cells were maintained in the incubator between measurements)

Dose-Responsive Bioluminescent Dynamics

- Because cells were kept intact and attached to the growing surface during measurements, the same cell population can be monitored repeatedly throughout the course of exposure
- A significant change in bioluminescence (p < 0.05) was observed 3 days after exposure to concentrations of E₂ as low as 1 pM

Comparison of the lux System with Current Sensor Technologies

- Produces non-specific background signal
- Sensitive to light-based technology

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