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SUSTAINED AUTOBIOLUMINESCENCE IN GLIOBLASTOMA CELLS VIA MICROFLUIDIC CULTURE AND SYNTHETIC PROMOTER OPTIMIZATION

Glioblastoma multiforme (GBM) is the most aggressive and lethal primary brain malignancy, characterized by diffuse infiltration, treatment resistance, and profound molecular heterogeneity. Despite intensive trimodal therapy—surgery, radiation, and temozolomide—median survival remains just 15 months, underscoring the need for more predictive models and novel therapies. Bioluminescence imaging (BLI) is a biotechnology for tracking tumor progression in vivo, but conventional systems require repeated luciferin administration, limiting consistency and temporal resolution. To overcome this, we engineered a codon-optimized, self-illuminating synthetic lux operon (pLux) for stable autobioluminescence in mammalian cells, eliminating substrate dependence.

To enable early, ethical, and scalable testing of such biosensors, we developed a microfluidic tumor-on-a-chip platform that offers a cost-effective, preclinical validation of the autobioluminescent cell models. We designed a microporous membrane-based microfluidic bioreactor—referred to as a microfluidic tumor reactor—to serve as a continuous perfusion system for autobioluminescent glioblastoma cells. This platform was developed to overcome confounding premature downregulation of bioluminescent signals observed in conventional static culture systems. Signal downregulation was hypothesized to arise from microenvironmental deterioration, particularly nutrient depletion and/or metabolic waste accumulation, conditions that may contribute to transcriptional silencing or degradation of the pLux cassette. By enabling continuous perfusion and selective molecular exchange across a semipermeable membrane, the microfluidic reactor maintains a more physiologically relevant nutrient-waste balance and supports long-term culture viability to resolve reporter signal fidelity.

With the observations in microfluidics, the existing viral origin (CMV) promoter in our synthetic lux construct, was exchanged for a chicken β-actin (CAG) promoter to drive synthetic lux expression. The CAG promoter was amplified to generate the construct pCAG-Lux in E.coli, it was then isolated, transfected, and tested in GBM cells to achieve more than three-times the duration of autobioluminescence in vitro.

Topical Area

Biology and life sciences

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