Tissue engineered colorectal cancer microspheres for drug screening

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Three-dimensional (3D) disease models have garnered widespread interest for use in later stages of the drug discovery process, such as preclinical efficacy and toxicology studies, due to their pathophysiologically relevant properties. However, there is a need and opportunity for 3D cancer models to be used earlier in the drug screening process. To meet this need, the 3D models must strike a balance between throughput, which includes scalability and uniformity, and physiological relevance, such as the ability to modulate key attribute of the tumor microenvironment.

Here we report the creation of 3D colorectal cancer (CRC) tissue models, referred to as VivoSpheres, and demonstrated their relevance to cancer drug screening. The VivoSphere production platform couples tissue engineering toolkits with microfluidics, enabling the scalable production of engineered cancer microspheres. The model supports the long-term maintenance of the cancer cell phenotype. In a preliminary study, we were able to generate more physiologically relevant drug responses.

We formed CRC VivoSpheres by encapsulating HT-29 CRC cells within poly(ethylene glycol)-fibrinogen hydrogel microspheres using our previously developed microfluidic platform. CRC VivoSpheres were rapidly produced with high cell densities (20×10^6 cells/ml) and high uniformity on day 0 with a coefficient of variation (COV) < 7%. This high uniformity was maintained for 15 days (COV ≤ 10%), which is critical for long-term dose studies. The cells maintained high viability and showed high proliferative capability with a significant increase in colony size and expression of Ki67 up to day 29. The encapsulated cells maintained the CRC phenotype over time with the expression of CD44 (cancer stem cell marker) and CK20 (CRC marker).

After establishing shipping conditions that maintained cell viability for remote use, the HT-29 VivoSpheres were shipped to the oncology team at Southern Research for drug testing. The CRC VivoSpheres were treated with DMSO, GANT61, and SRI-38832, the latter two of which are GLI1 inhibitors. Phase contrast images and western blot were used to assess the response of CRC VivoSpheres to the treatments. Oncogenic GLI1 transcription activity and NBS1 overexpression have been found to contribute to chemotherapeutic resistance, negating the anti-tumor effects of 5-fluorouracil. While 2D cultured HT-29s responded to treatment with GANT61, HT-29 VivoSpheres continued to express NBS1 following GANT1 treatment, but downregulated NBS1 in response to the GLI1 inhibitor SRI-38832, which is the same response Southern Research has seen in *in vivo* tumor models.

In conclusion, we have developed tissue-engineered 3D CRC models that hold promise for use in drug screening. These models have demonstrated an initial capability to reproduce the CRC phenotype and mimic *in vivo* drug response.