Establishment of In Vitro 2D Cell Cultures and 3D Organoids from Canine Intestinal Tumor

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Introduction and Objectives: *In vitro* models play a pivotal role in intestinal cancer studies, however, canine intestine cells are often challenging to grow in conventional 2D cell culture systems and often lack the structural complexity and microenvironmental cues of *in vivo* tumours. To address these limitations, 3D organoid cultures have emerged as a platform that preserves key features of the original tissue, including cellular heterogeneity, spatial organization, and functional characteristics. In this study, our aim was to establish both 2D monolayer cultures and 3D organoids from a canine intestinal tumour sample surgically harvested. This protocol enables the generation of stable, expandable canine-derived models, providing a valuable tool for comparative oncology, drug screening, and the investigation of tumour-specific pathways.

Materials and Methods: Fresh tumoral intestinal tissue was initially washed with PBS and cut into small fragments of $1mm^3$ to be enzymatically digested by the enzymes DNASE I 0,1mg/mL, Dispase 1mg/mL and Collagenase IV 0,5mg/mL for 1h incubated at 37ºC to obtain a viable cell suspension. After centrifugation at 400*g* 10mins 4ºC, cells were then seeded at 3,88 x 10^6 cells/mL in a t-flask coated with Matrigel to form a 2D monolayer supplied with DMEM medium 10% FBS, as well as seeded in Matrigel drops overlayered with optimized intestinal organoid culture medium to promote three-dimensional structure. Cell growth was monitored at 24H, 48H and 96H by microscopy and a growth curve was obtained through MTT assay.

Results: In the 2D culture system, cells exhibited a predominantly fibroblastic morphology and reached approximately 90% confluence after 96 hours, resembling typical epithelial-like morphology often reported for cells derived from canine intestinal tumours. Organoid structures began forming at 48 hours and reached morphological maturity by 96 hours, exhibiting organoid architecture resembling crypt buds and lumen enclosure, indicating successful epithelial organization. MTT assay results showed over 100% growth at 24 hours, indicating strong early proliferation, with continued expansion reaching maximal capacity by 72 hours.

Conclusions: We have successfully established a protocol of extraction and culture of canine intestinal tumour cells for both organoids and 2D cell culture. This method may provide an adequate and more reliable preclinical model to investigate tumorigenesis mechanisms and develop new treatments for both veterinary and human medicine.

Keywords: Intestinal tumour; 2D cell culture; organoids; veterinary oncology; in vitro model