

The challenge of culturing human colorectal tumor cells: establishment of a cell culture model by the comparison of different methodological approaches

Alessandra Failli¹, Rita Consolini¹, Annalisa Legitimo¹, Roberto Spisni², Maura Castagna³, Antonella Romanini⁴, Gaetano Crimaldi¹, and Paolo Miccoli²

¹Department of Medicina della Procreazione e dell'Età Evolutiva, Laboratory of Immunology, University of Pisa, Pisa; ²Department of Surgery, UO Surgery 2, University of Pisa, Pisa;

³Department of Surgery, U Anatomia Patologica III, University of Pisa, Pisa; ⁴Medical Oncology Unit, S. Chiara Hospital, Pisa, Italy

ABSTRACT

Background. Because colorectal cancer is a significant cause of morbidity and mortality in the Western population, knowledge of the molecular and biological alterations associated with its development is important. Since primary human colon cancer cultures from fresh tumor tissue are technically difficult to obtain, experiments in most laboratories are performed on colon epithelial cell lines, but these represent just one stage of tumor progression. Only primary cultures of neoplastic colonocytes may reflect the actual responsiveness of tumors at certain developmental stages to antitumor agents.

Methods. This paper analyzes several critical points concerning primary cultures, ranging from cell isolation to culture conditions, and compares different methodological approaches to isolate and cultivate a pure fraction of viable tumor cells. Samples of resected colorectal cancers were collected from 20 patients (stage T3 or T4). We compared *in vitro* several approaches of tissue disaggregation including mechanical disaggregation and enzymatic dissociation with trypsin or collagenase. Isolated cells were maintained in a short-term serum-free culture system. Evaluation of the purity and tumoral nature of isolated cells was performed by immunochemistry.

Results. We established the antibiotic concentration necessary during transport and washing of the specimens to prevent microbial overgrowth. We demonstrated that the number of viable cells was dependent on the dissociation method used. Mechanical disaggregation is not a valid dissociation method because of the high mortality of cells and might be used only in samples for molecular analysis. Comparison of the enzymatic digestion procedures showed that digestion with trypsin allowed the highest recovery of viable cells.

Conclusion. In this paper we analyzed several critical aspects of cell culture procedures and designed a methodological approach suitable for functional studies of colorectal cancer.

Key words: colorectal cancer, methodological approaches, primary cultures.

The work was conducted at the Laboratory of Immunology of the Department of Medicina della Procreazione e dell'Età Evolutiva, University of Pisa, Italy.

Correspondence to: Rita Consolini, MD, Department of Medicina della Procreazione e dell'Età Evolutiva, Pediatria 1, University of Pisa, Via Roma 67, 56100 Pisa, Italy.
Tel +39-050-992222;
fax +39-050-888622;
e-mail rita.consolini@clp.med.unipi.it

Received June 6, 2008;
accepted October 28, 2008.