DIFFERENTIATION OF HUMAN COLON CANCER CELLS

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INTRODUCTION

As it is the case for many tissues, there has been an increasing interest over the last decade in the use of cell cultures for studies related to the functions of the intestinal epithelium. These studies have been essentially performed with either organ cultures or primary cultures\(^1,2\). However there are some limitations in the application of such systems as they are difficult to manipulate, do not allow reproducible dynamic studies, and are not homogenous. An ideal tool would be the use of established differentiated cell lines originating from normal tissues. However, and despite attempts from a number of laboratories\(^1,2,3\), it has not been possible to establish such cell lines so far. In fact, the only cell lines that have been established have regularly failed to express any of the characteristics of terminal differentiation which would make them useful for studies related to the physiological functions of the intestinal epithelium\(^1,2,3\). This failure has been however circumvented by the finding that cell lines established from human colon carcinomas are able to express in culture most of the differentiation characteristics and functions normally associated with the human intestinal epithelium\(^1,2,4,5,6\). As appropriate as these cell lines may be for studying a number of functions related to the intestinal epithelium, it must be emphasized that, although these cells are closely similar to, and share a number of physiological properties with intestinal cells, they are not small intestinal, but colonic cells, and are not normal, but malignant cells. In order to further understand why such cell lines would express such differentiation characteristics it appears important to recall some of the cellular differentiation characteristics of colon cancers: by cellular differentiation should be meant the ability of some colon cancer cells to exhibit the ultrastructural morphology of either columnar absorptive cells, or mucus secreting cells, and to express proteins normally associated with the functional differentiation of the corresponding cells. Based on such a definition a variable proportion of differentiated cells are encountered in most colon cancers. Concerning absorptive cell types they may express not only brush border-associated proteins such as those normally associated with the colon, like CEA\(^7\), or villin\(^8\), but also brush border-associated hydrolases\(^9\) (sucrase isomaltase, aminopeptidase N, dipeptidylpeptidase IV, alkaline phosphatase) which in this case appear to be closer to the enzymes present in the fetal colon than to those expressed in the small intestine\(^10,11,12\). Concerning mucus secreting...
cells they can be of two types depending on the organ specificity of secreted mucins, some being of the colonic type and others being of the gastric type. In addition to columnar absorptive and mucus secreting cells, a third type of cells can be found in colon cancers namely cells which are polarized but do not express an apical brush border and do not secrete mucins. With regard to these different cell types it is not surprising that within cell lines established from such cancers some cells would express the differentiation characteristics which preexist in the original tumors. Indeed analysis of established cell lines shows that the same heterogeneity of differentiation which is found in colon cancers also occurs in cultured cell lines. Accordingly, and with the exception of these cell lines in which no differentiated cells can be found (which represent 50% of the cell lines tested so far), colon carcinoma cell lines can be divided into three groups: Group 1 includes cell lines in which 100% cells are differentiated and express a unique differentiation phenotype; Group 2 includes cell lines which are heterogenous as not all the cells are differentiated and not all the differentiated cells express the same type of differentiation; Group 3 includes cell lines which express neither a columnar cell absorptive type nor a mucus secreting type but which are able to form a polarized epithelium (references to cell lines cited in this article are reviewed in 15).

GROUP 1: THE CACO-2 CELL LINE.

The Caco-2 cell line is the only known example of a cell line belonging to this group. It was established in 1974 by Dr. Jorgen Fogh (Sloan Kettering Memorial Cancer Center, Rye, NY). The original tumor had been removed from a 72 year-old male patient of blood group O. It is interesting to note that prior to the removal of the tumor this patient had been treated by 5-fluorouracil and cytoxan (J. Fogh, personal communication). Since the first observation that Caco-2 cells in culture were able to undergo a complete and terminal differentiation of the columnar absorptive cell type (Fig. 1) a considerable number of laboratories have been using this cell line (for review, see 2,4,6). Because of the amount of literature already available for this cell line, only the main characteristics of differentiation of the cells will be reported here as well as some details which may be of interest for future utilizers of this line.

A first characteristic of the differentiation of the cells is that it is a growth-related process: the cells, although already polarized in the exponential phase, are not differentiated; the process of differentiation starts after the cells have reached confluency and is complete after one week (i.e. on day 16 when cells are seeded at a density of 1x10^4 cells/cm^2).

A second characteristic which is unexplained so far is that the degree of differentiation of the cells, as appreciated by the level of enzyme activities, increases with the number of passages by 20: cells obtained from either the Sloan Kettering Memorial Cancer Center or the ATCC are usually at passage 16; in our experience the degree of differentiation increases progressively until passage 70 and then remains stable (at least until passage 150, which is the latest passage tested in our laboratory).

The differentiation characteristics of post-confluent cells have been extensively reported and will be just summarized: whether cultured on impermeable support (plastic or glass) or on semi-permeable filters post-confluent cultures form a true monolayer of polarized cells which exhibit at their upper surface a typical brush border and at the