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Review

What are the best routes to effectively model human colorectal cancer?

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ABSTRACT

Colorectal cancer (CRC) is the third most common cancer in the UK, with over 37,500 people being diagnosed every year. Survival rates for CRC have doubled in the last 30 years and it is now curable if diagnosed early, but still over half of all sufferers do not survive for longer than 5 years after diagnosis. The major complication to treating this disease is that of metastasis, specifically to the liver, which is associated with a 5 year survival of less than 5%. These statistics highlight the importance of the development of earlier detection techniques and more targeted therapeutics. The future of treating this disease therefore lies in increasing understanding of the mutations which cause tumourigenesis, and insight into the development and progression of this complex disease. This can only be achieved through the use of functional models which recapitulate all aspects of the human disease. There is a wide range of models of CRC available to researchers, but all have their own strengths and weaknesses. Here we review how CRC can be modelled and discuss the future of modelling this complex disease, with a particular focus on how genetically engineered mouse models have revolutionised this area of research.

1. Colorectal cancer: pathways and progression

The development of sporadic CRC is a multistep process in which an accumulation of genetic mutations leads to the progression from normal intestinal epithelium to dysplastic tissue to benign adenoma through to metastatic carcinoma. Studies comparing human tumour tissue to normal tissue have highlighted many key mutations which are commonly involved in colorectal tumourigenesis, discussed below, and this knowledge has been pivotal to the development of many of the available models of CRC.

Based on studies of the frequency of gene mutations at various stages of progression in human tumours, Fearon and Vogelstein (1990) proposed a model whereby loss of function of *Adenomatous polyposis coli* (APC) initiates the formation of a benign lesion, followed by an activating mutation in KRAS, allelic loss of the 18q locus and mutation of p53, which all contribute to the progression to malignant disease. This model has now been further explored in order to ascertain the importance of timing of mutations on tumour progression, and found that interestingly, it is surprisingly rare for KRAS and p53 mutations to co-exist within a tumour, hinting at

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the complexity of the nature of colorectal tumourigenesis as they drive different pathways to tumour progression (Smith et al., 2002).

The technique of deep sequencing has enabled the identification of a range of drivers of colorectal tumourigenesis, leading to further refinement of the Fearon–Vogelstein model. In order to identify common mutations and drivers of CRC, deep sequencing has been used to uncover genes altered between normal and tumour tissue in a small number of colorectal cancers. Once a number of potential tumour driving mutations was identified, the genetic status of these genes could then be analysed in a much larger number of samples (Wood et al., 2007). Despite the clear identification of common loss of function mutations in APC, p53 and activating mutations in KRAS, the authors present the idea that a range of other less common mutations contributes to the tumour phenotype by providing a small fitness advantage to the tumour via disruption of a few key pathways.

APC is a tumour suppressor which functions by negatively regulating the Wnt-signalling pathway. Mutations in APC result in constitutive activation of the canonical-Wnt-signalling pathway leading to a build up of nuclear β-catenin and have been found in more than 80% of all sporadic human CRC tumours. Nuclear β-catenin functions as a transcription factor for a range of Wnt-target genes such as C-myc and Lef1. Wnt-target genes have a variety of functions, but most play some role in the control of essential homeostatic processes such as mitosis, apoptosis and migration, misregulation of which can result in cancer.

More than 5% of all cases of CRC are due to inherited disorders, most commonly Familial Adenomatous Polyposis (FAP) and Hereditary Non-Polyposis Colorectal Cancer (HNPCC). These familial forms of CRC can teach us a great deal about the genes involved in tumourigenesis and it is interesting to note that sufferers of Familial Adenomatous Polyposis (FAP) carry a heterozygous mutation for APC and a significant number of colorectal tumours from HNPCC patients display somatic mutations of APC highlighting the importance of APC as a tumour suppressor within the colon.

In order to understand the complex nature of CRC, including how it develops and how it should be treated, it is essential to use models which recapitulate the human disease as closely as possible. CRC patients present most frequently with few large tumours located in the large intestine, which are capable of invasion and metastasis. Modelling of CRC must therefore take these criteria into account.

2. Different approaches to modelling CRC

Patients present with CRC at a range of stages of the disease, and the combination of mutations which can promote the disease is huge, which make it a difficult disease to model accurately. In order to develop therapeutics for this complex disease it is necessary to understand which mutations are key to tumour progression, why some tumours respond better to certain therapies than others and the cellular processes which are necessary for disease progression which could be potential drug targets.

2.1. *Human cell lines*

Cell lines established from tumour tissue derived from patients with CRC are widely used to model the disease, and there are a number of established cell lines which are readily available for this purpose. As these cell lines are obtained from human patients, they are directly relevant to human disease and cell culture techniques enable high throughput experiments which are quick and relatively inexpensive. This makes cell culture an attractive model system for the disease, and are therefore widely used.

By mimicking tumour behaviour in culture, certain characteristics of the tumour cells can be identified, such as properties which represent the cells ability to aggregate, migrate and invade. These properties can be quantified and any changes assessed when the cells are exposed to a range of therapeutics. For example, a range of CRC cell lines (HCT-116 *p53* wild type, HT-29, HCT-116 *p53*−/− and SW-620 cells) was used by Nautiyal et al. (2010) in order to assess the combined effects of curcumin and dasatinib on the ability of the cells to form colonies, invade through an extracellular matrix and the formation of tubules by endothelial cells. These readouts are commonly used as an indicator of metastatic capabilities, and in this study the enhanced inhibition of "metastasis" when the therapies were used in combination was confirmed in an *in vivo* model. This study not only highlights the utility of cell lines as a model for CRC, but also addresses the downfalls of the system by comparing a range of different cell lines and supporting the data with an *in vivo* model.

One major disadvantage of using cell lines is that the important interaction of the tumour cells with their environment is missing. The artificial environment in which these cells are maintained can not only radically alter the epigenetics of the tumour, but also many of the complex 3-dimensional interactions between cells are lost. For example, by culturing cancer cells in a 2-dimensional plane, the Oxygen levels between cells are kept relatively equal, which may be an unrealistic scenario within a growing tumour. The major limitation of cell culture is its inability to model the effects of organism response to a tumour, such as immune response and angiogenesis, two factors which are known to exert a large influence on the tumour development. Despite these drawbacks, culturing of a variety of CRC cell lines plays an important part in extending knowledge of genetic involved in CRC and the response of tumour cells to drugs and very frequently enables the elucidation of a mechanism of a therapy which is known to work *in vivo*. However, ideally, CRC needs to be modelled *in situ* in order to best represent the tumour environment and behaviour.

2.2. *Xenografts*

One attempt to overcome the limitations of cell culture is through the use of xenografts. For over thirty years, xenografts, whereby murine or human tumours are implanted into recipient mice, have been used to generate models of human CRC progression and metastasis, to great effect. Tumour cell lines or cell suspensions can be injected subcutaneously, directly into the colon or cecum, into the portal vein or via intraperitoneal injection. Cultured cells can be injected