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Viable intraluminal tumour cells and local/regional tumour growth in experimental colon cancer

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Summary

To determine if viable intraluminal tumour cells can leak through a watertight anastomosis and cause local/regional (extraluminal) tumour growth, tumour cells were introduced 2 cm proximal to a colonic anastomosis following laparotomy in a Wistar/Furth rat colon cancer model. Local/regional tumour growth was observed in all rats except a sham anastomotic group. No intraluminal tumour growth was observed in either group. Viable intraluminal tumour cells cause local/regional tumour growth by leakage through a clinically intact anastomosis and may be an important cause of local/regional tumour growth in human colorectal cancer.

Introduction

Local/regional tumour recurrence, ie recurrence at or in the region of an anastomosis, in abdominal wounds or drain sites is the most common cause of tumour recurrence following 'curative' resection of a primary colorectal cancer. The most direct evidence for this comes from the Minnesota re-operative series where 48% of recurrences in patients re-operated upon at 6–12 monthly intervals following 'curative' resection of a Dukes' B or C rectal cancer were local/regional recurrences alone *(1)*. Only 8% were due to distant metastases alone, while 44% were due to distant metastases and local/regional recurrences combined. A similarly high incidence of local/regional tumour recurrence was noted in the Large Bowel Cancer Project, a prospective study involving over 4000 patients with colorectal cancer *(2)*. The possible causes of such local/regional tumour recurrence might include the following:

1 An inadequate resection of the primary tumour.
2 The development of metachronous primary tumour in the region of the anastomosis.
3 The promotion of carcinogenesis by suture material at the anastomosis *(3,4)*.
4 Implantation of viable tumour cells, present, in lymphatics, in blood vessels, in the peritoneal cavity, or intraluminally as viable exfoliated cancer cells from the primary cancer.

Recent work from Umpleby *et al. (5)* demonstrated viable exfoliated intraluminal tumour cells in 70% of 74 colon cancer specimens. Viable cancer cells were found proximal to carcinomas in 57% of cases and distal in 84% at distances as great as 35 cm from the primary tumour. While one may postulate that the presence of large numbers of viable tumour cells have a role in the aetiology of suture line or anastomotic recurrence, such recurrences in human colorectal cancer are rare. However, should such cells leak or permeate through an otherwise clinically intact anastomosis and give rise to local/regional tumour recurrence, their presence would be of much greater clinical importance. The aim of this study was to develop and test a suitable model to determine if viable intraluminal tumour cells could leak through an otherwise intact anastomosis and cause local/regional tumour growth.

Materials and methods

ANIMALS

Male Wistar/Furth rats, 6–8 weeks old, were obtained from Harlan Industries, Madison, Wisconsin. All animals were housed in a temperature (22±1°C) and light cycle (12 h light, 12 h dark) controlled room. All were allowed free access to food and drinking water.

TUMOUR ISOGRAFT

The DMH-W163 tumour isograft was kindly provided by Dr Glenn Steele Jr, New England Deaconess, Boston Ma. This is a poorly differentiated mucin producing adenocarcinoma explanted from a dimethylhydrazine colon cancer in a Wistar/Furth rat. Isograft was maintained by serial passage of tumour subcutaneously in

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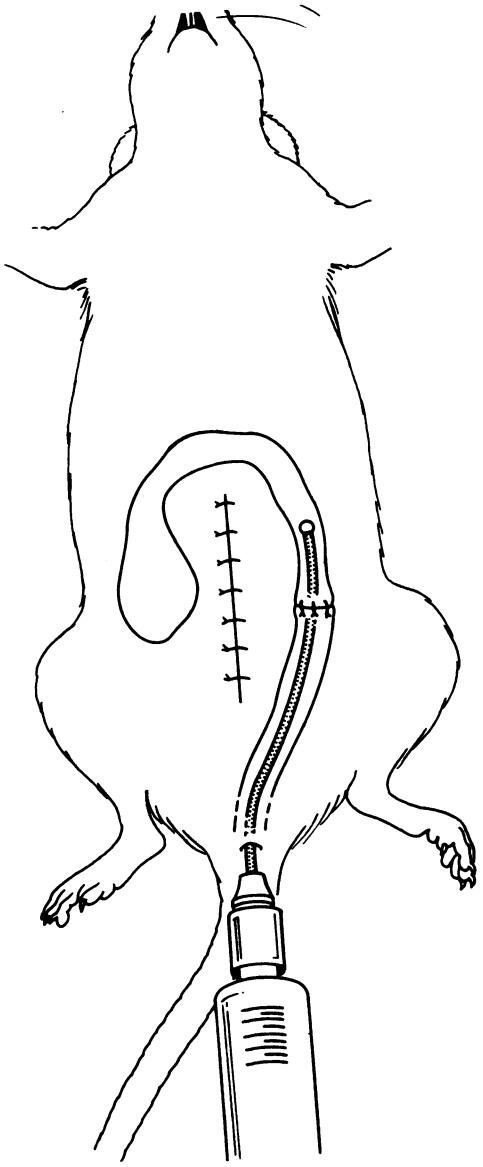

FIG. 1 Model used in study.

syngeneic recipients' inguinal regions. Single cell suspensions were obtained from this isograft by passage of solid tumour through a sterile 60-mesh stainless steel screen, washing three times in phosphate buffered saline (PBS) and resuspending in PBS at a concentration of 5×10^6 viable cells/ml.

ANIMAL MODEL

The animal model used in this study is illustrated in Fig. 1. All animals had their descending colon divided and an anastomosis performed using a single layer of 8–10 interrupted sutures under ether anaesthesia. A cannula was then introduced per rectum, the tip of which was placed 2 cm proximal to the anastomosis. Anastomoses were checked to be *watertight* by distension with sterile water. Laparotomy incisions were closed with a continuous suture and skin clips. One millilitre of the tumour cell suspension (5×10^6 viable cells) was then slowly passed through the cannula. The cannula was withdrawn and the animal was allowed to recover from anaesthesia.

EXPERIMENTAL DESIGN

Three groups of rats were used in this experiment, one ($n=11$) in which all anastomoses were performed using 4/0 prolene, one ($n=11$) in which all anastomoses were performed using 4/0 silk. A further group ($n=4$) underwent laparotomy and sham anastomosis under the same experimental conditions to exclude the possibility of intraperitoneal contamination with tumour cells at operation.

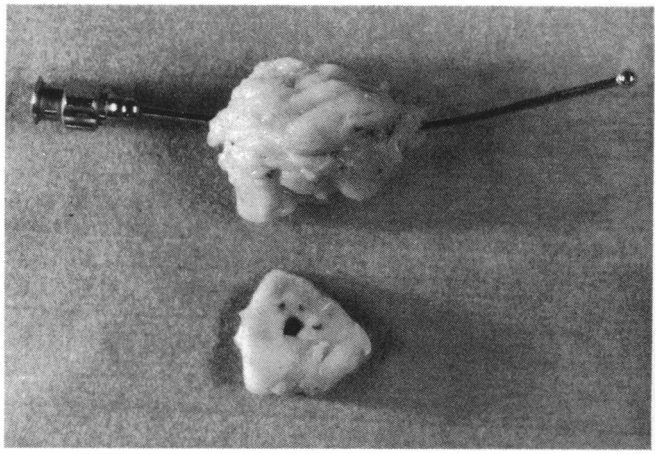

FIG. 2 Macroscopic appearance of anastomosis demonstrating a patent lumen with perianastomotic tumour growth.

Results

No animal died postoperatively in this study and all had recovered fully 24–48 h following operation. After 3–5 weeks following operation all rats except the sham group had obvious ascites with palpable intra-abdominal tumour. These animals were killed and tumour was confirmed histologically. All anastomoses were patent and intact (Fig. 2), and no evidence of intra-abdominal sepsis or abscess formation was observed in any animal. Tumour was distributed around the anastomosis, with seedlings on visceral surfaces, omentum and abdominal wound. No intraluminal tumours were noted. All animals in the sham group were killed 3 months later and had no evidence of intraluminal or intra-abdominal tumour.

Discussion

Viable intraluminal tumour cells from a poorly differentiated mucin producing colon adenocarcinoma were found to permeate through an otherwise clinically intact anastomosis and cause local/regional tumour growth in a rat model in this study. Histological section through anastomoses demonstrated perianastomotic tumour with infiltration of bowel wall with carcinoma. No intraluminal tumour growth was observed. In 1968, Yu and Cohn *(6)* claimed similar results in an experimental rabbit model. However, in this study it could not be confirmed if the local/regional tumour growth observed was related to contamination of the peritoneal cavity with tumour cells at the time of installation or from cell permeation through a clinically intact suture line. In their model, intraluminal tumour cells were introduced by injection through the wall of the terminal ileum and then closing the defect with a pursestring suture. A previous 2 cm colotomy incision had been repaired in two layers in all animals. In the present study, tumour cells were introduced proximal to the anastomosis following closure of the laparotomy incision, thus avoiding any possible contact of tumour cells with the peritoneal cavity. In addition, to exclude the less likely contamination of the

peritoneal cavity (as aseptic technique was observed) by tumour cells on gloves and instruments with subsequent tumour growth, a group of rats underwent laparotomy and sham anastomosis under the same experimental conditions.

While there can be little doubt that an adequate resection is of importance in avoiding local/regional tumour recurrence when treating primary colorectal cancer, this and recent studies serve to emphasise the contribution of viable tumour cells to such recurrences *(5,7)*. The ability of viable tumour cells to implant and cause local tumour growth is well described in the human situation *(8)*. Indeed the injury sustained to tissues when resecting a primary colorectal cancer would seem to provide a suitable 'soil' for viable cells to adhere and grow. In addition, tumour growth may be promoted by certain suture materials acting as a nidus for tumour growth *(9)*.

Since there is a wide variability in local/regional recurrence rates reported among different surgeons *(2)*, it becomes clear that surgical technique is an important factor in determining incidence rates for this problem. It appears likely that many surgeons underestimate the contribution of the factors mentioned in causing such recurrences and thus take few preventive measures. This is unfortunate since while local/regional recurrence has been shown to be amenable to resection in up to 50% of cases at a second operation, it is rarely accompanied by long-term survival *(10)*. Of 67 asymptomatic patients in our CEA-directed second-look surgery programme undergoing a second operation for recurrent colorectal cancer, only 16 (24%) survived 5 years or longer. Moreover, most of these survivors were from patients who had hepatic secondaries resected 10 (15%) and not from patients with local/regional recurrences, where only 6 (9%) survived long-term.

Though this model is experimental and performed with a highly virulent colonic carcinoma, it nevertheless demonstrates tumour cells' ability to migrate across an anastomosis and implant in sufficient numbers to cause perianastomotic tumour growth. While there can be little doubt that such an event is also possible in human colorectal cancer, the significance of its contribution to local/regional tumour recurrence is not known. It remains necessary, therefore, to compare local/regional tumour recurrence rates and overall survival in patients with and without viable intraluminal tumour cells at anastomotic ends in future clinical trials with colorectal cancer.

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