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# Twelve colorectal cancer cell lines exhibit highly variable growth and metastatic capacities in an orthotopic model in nude mice

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**Abstract**

Orthotopic tumour models for colorectal cancer are a complementary tool for the study of tumours *in vivo*. They are more closely related to human cancer than are subcutaneous tumour models, since evaluation of spontaneous metastasis formation is possible. In the present study, fragments of subcutaneous xenografts established from 12 well-described and generally available colorectal cancer cell lines were implanted in the caecum of nude mice and tumour growth and metastatic events registered. The results showed considerable differences between the cell lines with respect to take rate, tumour growth and metastatic ability. This resulted in variable disease progression that seemingly reflects clinically relevant heterogeneity. The most common metastatic findings were mesenteric lymph-node metastases, occurring at variable frequency in tumour-bearing mice with 10 out of 12 cell lines, whereas only one line gave rise to liver metastases, in two of 10 animals. The study provides useful background information on the 12 colorectal cancer cell lines in a clinically relevant orthotopic tumour model.


*Keywords:* Cell lines; Colorectal cancer; Orthotopic tumour model

## 1. Introduction

Human colorectal cancer xenografts grown subcutaneously in immunodeficient mice provide important experimental models for acquiring knowledge about biological tumour properties and drug response. A limitation, however, is the lack of metastasis from the subcutaneous site, rendering the model inappropriate for investigating the spontaneous metastatic process. For this reason, orthotopic tumour models have been developed utilising injection of colorectal tumour cells or implantation of tumour tissue in the caecum. Such models have been found to be of relevance in portraying the human metastatic process [1–4]. Tumours growing orthotopically also have different sensitivities to chemotherapy compared to subcutaneously growing tumours [5]. Consequently, the orthotopic techniques provide a complementary tool that enables the study of spontaneous tumour metastasis in a model more closely related to human colorectal cancer.

The orthotopic approach has been successfully used to implant or inject material directly from patient's tumours, making it possible to study tumours that have not been altered by years of culture *in vitro* and *in vivo*. Conversely, many commonly used cell lines have been extensively characterised in various *in vitro* and *in vivo* systems, including invasive and metastatic properties, genetic aberrations, drug response and expression of cancer-associated genes. Because of the accumulated knowledge, these well-described cell lines are attractive for use in orthotopic tumour models, provided that cell lines relevant for the purpose of the study are selected.

The purpose of the present study was to test a large panel of generally available human colorectal cancer cell lines in an orthotopic model to determine which lines would be best suited to investigate the process of spontaneous metastasis formation. Our results showed

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considerable differences between the cell lines with respect to take rate, growth rate and metastatic ability, resulting in a panel of tumours that seem to reflect a heterogeneity that may be clinically relevant.

## 2. Materials and methods

### 2.1. Cell lines

Twelve CRC cell lines were used, and in Table 1 the known characteristics of each line are listed. The Co205, HCT15, HCT116, Colo320DM, SW480, SW620, CaCo2, HT29 and WiDr cell lines were all purchased from ATCC (Manassas, VA). KM20L2 and HCC2998 were kindly provided by Michael R. Boyd (National Cancer Institute, FCRF, Frederick, MD). Co115 cells were obtained from B. Sordat, Epalinges, Lausanne, Switzerland. The cell lines were cultivated in RPMI with 10% v/v fetal calf serum under recommended conditions, and the cells were detached with trypsin–EDTA (all products from Gibco, BRL Life technologies, UK). All cell lines were negative for mycoplasma infection.

#### 2.1.1. Animals

Locally bred female BALB/c nude (nu/nu) mice, 6–8 weeks of age at implantation, were used. The animals were maintained under specific pathogen-free conditions, and food and water were supplied *ad libitum*. Housing and all procedures involving animals were performed according to protocols approved by the animal care and use committee, in compliance with the National Committee for Animal Experiments guidelines on animal welfare. Mice were anaesthetised with 0.1 ml/10 g of a mixture of equal parts of fentanyl/fluanison (Hypnorm; Janssen, Beerse, Belgium) and midazolam (Dormicum; Roche, Basel, Switzerland) during surgical procedures. After surgery each mouse was given one subcutaneous injection of 3 μg buprenorphin hydrochloride (Temgesic; Schering-Plough, Kenilworth, NJ) for postoperative pain relief.

#### 2.1.2. Tumour models

Subcutaneous xenografts were established by injection of $1 \times 10^6$ tumour cells in both flanks of 2–3 animals per cell line and the growth was monitored regularly. Tumours were selected for orthotopic implantation at an average size of 1 $cm^3$ and tissue was cut into $2 \times 2 \times 2$ mm pieces. Superficial regions of the tumours were used for implantation and all sampled tumours were examined histologically to confirm that areas of the tumour corresponding to the sampled regions contained viable tissue. Implantation was performed according to the method described by Pocard and colleagues [6] with some modifications. In brief, the caecum was exteriorised through a small midline laparotomy and a piece of tumour tissue sutured to the caecal surface with a single Maxon 7/0 suture, leaving the tumour tissue buried in a 'pouch' consisting of a double caecal wall on each side. After implantation, the abdominal wall was closed in two layers with Dexon 5/0.

Each cell line was implanted orthotopically in series of approximately 10 mice. However, for the first two cell lines tested, Colo320DM and HCT-15, two such series were performed, since systematic histological evaluation of the lymph nodes had not been performed in the first series.

#### 2.1.3. Sampling and evaluation

The well being of the mice was carefully monitored and animals were killed when and if signs of disease were detected. Healthy mice were observed for 18–41 weeks before being killed. Autopsy was performed and macroscopic assessment was made for the presence of primary tumour, lymph-node or distant metastases. All detected macroscopic lesions were measured and sampled for histological examination. Additionally, the mesenteric lymph nodes draining the caecal area were

Table 1
Cell lines used for orthotopic implantation

| Cell line | Derived from | Dukes' stage | Patient (sex/age) | Reference |
|---|---|---|---|---|
| Caco-2 | Primary colon cancer | –[a] | Male/72 | [13] |
| WiDr | Primary adenocarcinoma of the rectosigmoid colon | –[a] | Female/78 | [14] |
| HT-29 | Moderately differentiated primary colon cancer | –[a] | Female/44 | [15] |
| KM20L2 | Primary colon tumour | D | –[a] | [16] |
| HCC2998 | Well-differentiated primary colon cancer | –[a] | –[a] | [17] |
| Co 205 | Colon adenocarcinoma, metastatic site, ascites | D | Male/70 | [18] |
| HCT 116 | Poorly differentiated primary colon cancer | –[a] | Male/–[a] | [19] |
| Co115 | Poorly differentiated carcinoma from ascending colon | C | Female/77 | [20] |
| HCT-15 | Well- to moderately differentiated carcinoma of sigmoid colon | C | [a] | [21] |
| SW480 | Moderately differentiated adenocarcinoma of descending colon | B | Male/50 | [22] |
| SW620 | Lymph-node metastasis from colon adenocarcinoma | C | Male/51 | [22] |
| COLO 320DM | Moderately differentiated adenocarcinoma of sigmoid colon | C | Female/55 | [23] |

[a] Data not available.

sampled for histological examination if a caecal tumour was detected or suspected, even if no macroscopic lymph-node metastases were present.

Regular measuring of tumour size is impractical in this model, since the intra-abdominal tumour is not accessible without laparotomy. Consequently, we had to devise a different method to compare growth rates for different cell lines. The final tumour volume for each successful implantation was calculated by the formula $0.5 \times \text{length} \times \text{width}^2$. The tumour volume was divided by the time from implantation to sampling (weeks), and the resulting value was designated the growth index of that particular tumour.

## 3. Results

### 3.1. Take rate

Six of the 12 cell lines had take rates of 100% (KM20L2, HCT116, HCT15, SW480, SW620 and Colo320DM), whereas intermediate take rates were observed for Co115 (90%), HCC2998 (88%) and HT29 (69%). Tumours developed in approximately 40% of the mice implanted with CaCo2, WiDr and Co205 (Table 2). Histological assessment of the tumour tissue used for implantation showed no difference in the viability of tumour tissue that could explain the variation in take rate.

### 3.2. Growth pattern

All harvested tumours were examined by conventional microscopy of haematoxylin–eosin-stained sections to evaluate growth characteristics. All cell lines exhibited invasive growth in the murine caecal wall, some to the extent that there was penetration into the lumen of the caecum.

### 3.3. Growth index

The time between implantation and the appearance of disease symptoms in tumour-bearing mice varied considerably, as the animals were killed between 3 and 41 weeks post-implantation. The median values (Table 2) indicate differences between cell lines; additionally, for some of the series, large variation was also seen between animals implanted with the same cell line. The tumour volume at sampling differed similarly, as indicated by measured tumour volumes between 0.001 and 9 $cm^3$. These differences are depicted in Table 2, showing substantial variation in median growth indices between the orthotopic xenografts from the different cell lines. The lowest growth indices were observed for CaCo2 and WiDr, indicating that small tumours were harvested following a long growth period in the caecum.

Table 2
Detailed results for all orthotopic implantation experiments

| Cell line name | Number of mice implanted | Local tumour growth | | | | Metastatic findings in tumour-bearing mice | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Take rate | Time from implantation until sacrifice | Tumour size at autopsy | | Lymph node metastases | Liver metastases | Carcinomatosis | |
| | | Number of mice (%) | Median weeks (range) | Median volume in $cm^3$ (range) | Median growth index | Number of mice (%) | Number of mice | Number of mice (%) | |
| CaCo2 | 10 | 4/10 (40) | 24.9 (6.1–41.4) | 0.03 (0.001–0.11) | 0.005 | 1/4 (25) | 0 | 0/4 (0) | |
| WiDr | 9 | 4/9 (44) | 27.8 (20.7–38.1) | 0.08 (0.02–0.13) | 0.012 | 2/4 (50) | 0 | 0/4 (0) | |
| HT29 | 13 | 6/13 (69) | 12.1 (8.0–36.9) | 0.11 (0.08–0.45) | 0.035 | 5/6 (83) | 0 | 0/6 (0) | |
| KM20L2 | 10 | 10/10 (100) | 9.1 (7.0–9.1) | 0.08 (0.02–0.59) | 0.037 | 6/8 (75) | 0 | 2/8 (25) | |
| HCC2998 | 8 | 7/8 (88) | 13.0 (3.0–19.6) | 0.21 (0.03–0.67) | 0.065 | 6/7 (86) | 0 | 0/7 (0) | |
| Co205 | 9 | 4/9 (44) | 10.9 (10.0–11.9) | 0.18 (0.04–1.08) | 0.068 | 2/4 (50) | 0 | 0/4 (0) | |
| HCT116 | 10 | 10/10 (100) | 8.7 (6.0–9.1) | 0.26 (0.02–1.18) | 0.118 | 7/10 (70) | 0 | 2/10 (20) | |
| Co115 | 10 | 9/10 (90) | 9.1 (7.1–15.4) | 0.27 (0.09–1.15) | 0.119 | 5/9 (56) | 0 | 0/9 (0) | |
| HCT15 | 19 | 19/19 (100) | 10.0 (7.3–12.1) | 0.38 (0.02–1.86) | 0.152 | 9/13 (69)[a] | 0 | 3/19 (16) | |
| SW480 | 10 | 10/10 (100) | 8.9 (5.3–14.9) | 0.41 (0.13–1.08) | 0.185 | 0/10 (0) | 0 | 1/10 (10) | |
| SW620 | 10 | 10/10 (100) | 6.1 (5.3–12.0) | 0.57 (0.18–1.15) | 0.373 | 1/9 (11)[a] | 2/10 | 6/10 (60) | |
| Colo320DM | 19 | 19/19 (100) | 3.7 (3.1–9.3) | 1.28 (0.49–9.38) | 1.380 | 0/9 (0)[a] | 0 | 6/19 (32) | |

[a] Lymph-node examination by microscopy was not performed for all tumour-bearing mice in these series.

One cell line, Colo320DM, stands out with a very high growth index, and it must be noted that mice with such tumours, in contrast to those implanted with other cell lines, were killed because of increased abdominal volume, and not due to general symptoms from the tumour. The animals tolerated the large tumour burden from this cell line extremely well, to the extent that the first series of tumours with this cell line were allowed to grow to very large (2.5–9.4 $cm^3$).

### 3.4. Metastasis formation

In addition to local growth, the main findings were metastases to mesenteric lymph nodes draining the caecal area. In some cases, metastatic lymph nodes were discernible macroscopically, whereas histological examination was often necessary to determine whether tumour tissue was present in the lymph nodes. The xenografts produced lymph-node metastases in 0–90% of tumour-bearing mice (Table 2), with the highest frequencies for HCC2998 (6 of 7 mice) and HT29 (5 of 6 mice). Evidently, for the cell lines with low take rates (CaCo2, WiDr and Co205), the numerical basis for calculating a percentage is rather uncertain. Only one of the cell lines, SW620, gave rise to liver metastases, represented by multiple small (2–3 mm) metastatic nodules in the parenchyma of the left liver lobe in two of 10 animals, whereas another mouse had mesenteric lymph-node metastases (Table 2). Lung metastases were not detected in any of the animals. Another finding observed for half of the cell lines was peritoneal carcinomatosis, defined as tumour deposits attached to the peritoneal surface of the abdominal wall or to the surface of intra-abdominal organs distant from the primary tumour (Table 2). In most cases, multiple small nodules were observed, but single large tumours were also found.

## 4. Discussion

Twelve human colorectal cancer cell lines studied in an orthotopic murine model showed large variations in tumour take rate, growth index (tumour volume/time) and frequency of metastasis. Although the six cell lines with the most rapidly growing tumours all had take rates close to 100%, there was no strict correlation between take rate and growth index, as some relatively slowly growing tumours had high take rates and vice versa. Commonly, metastases were observed in mesenteric lymph nodes. However, whereas the three cell lines with the highest growth indices (Colo320DM, SW620 and SW480) produced lymph-node metastases in only one animal, with the other cell lines 20–90% of the mice had such metastases. It is not known whether this difference could be ascribed to differences between the cell lines in inherent metastatic properties, or that rapid tumour growth led to a tumour burden that caused disease symptoms before metastases had time to establish. Notably, one cell line (SW620) with a high take rate and growth index, but lymph-node metastasis in only one animal, was the single cell line that produced liver metastases. Thus, in two of 10 cases, multiple small metastases were found in the left liver lobe, possibly reflecting a higher metastatic potential or a preference for growth in liver tissue. Six of the 12 cell lines, all of which had take rates of 100%, produced widespread carcinomatosis. This might theoretically have resulted from contamination during the implantation procedure or, more interestingly, from shedding of tumour cells during growth, a possibility that could not be reliably examined.

In addition to the observed differences between the cell lines, highly variable results were seen within some of the series with respect to take, the elapsed time between implantation and killing, and the size of the harvested caecal tumour as well as the occurrence of metastatic findings. A probable explanation for this is that different pieces of tumour tissue are implanted in different individuals, and although attempts are made to standardise procedures, some biological variation will be seen in such experiments.

Objections might be raised to the choice of study design, particularly the use of the appearance of disease symptoms to define an endpoint for each animal. However, we had already observed highly variable growth rate of the cell lines in subcutaneous xenografts (data not shown), and it was reasonable to expect similar diversity in the orthotopic setting. An alternative approach of killing animals at a set time after implantation might not have disclosed the observed variation either between cell lines or between mice implanted with the same cell line. Our objective was to explore the growth and metastatic potential of each cell line, and using this strategy, we were able to follow the animals as long as possible without compromising ethical considerations.

Others, using injection of tumour cells and other techniques for transplanting tissue in the caecal wall, have employed different strategies for orthotopic studies of colorectal cancer. The present method has been used successfully with both xenografts and freshly biopsied tumour tissue [6,7], and is simple and rapid to perform. Each procedure takes on average 10 min and only standard surgical equipment is required. With this technique, the xenografts grow from the serosal surface of the caecum instead of originating in the mucosa, as colorectal cancer does in the patients. This suggests that with infiltrating tumours the exterior lymphatic drainage site would be reached at an earlier stage than in a clinical setting. Nevertheless, we found a typical infiltration pattern in the murine caecal wall, and lymph-node metastases occurred at the expected drainage site in the mesocolon, supporting the relevance of the model.

Kuo and colleagues [8] studied eight colon cancer cell lines (including Co205 and WiDr) in a similar orthotopic model, in which subcutaneous xenografts were sutured to the caecum following scraping of the serosal surface. They reported high take rates (100%) for all cell lines tested, while in our study the take rates for the Co205 and WiDr were rather low (40%). There is no obvious explanation for these observed differences, but they might be attributed to the common experience that cell lines may differ in characteristics when studied in different laboratories. The Co205 and WiDr lines did not give rise to liver metastases in any of the studies, and Kuo and colleagues categorised them as non-metastatic. However, whereas they did not report on lymph-node metastases, we found that both cell lines metastasised to mesenteric lymph nodes in two of four tumour-bearing animals.

Differences in results obtained *in vivo* with the same cell lines in different institutions might also be related to the use of different strains of mice. Thus, Guilbaud and colleagues [9] used several cell lines in a caecal implantation model similar to ours. Their results for untreated HT29 tumours implanted in Swiss nude mice were comparable to ours, whereas in SCID mice the HCT116 cell line produced both lymph-node metastases in four of five mice, as well as liver and lung metastases in two of five. Moreover, they reported high rates of carcinomatosis, in seven of eight and four of five animals with HT29 and HCT116 tumours, respectively. It is still unclear, however, to what extent the genetic composition of immunodeficient mice has a consistent and convincing effect on take rate and growth of human tumours [10].

In a study employing orthotopic implantation of nine different human primary colorectal tumours, highly variable growth rates were observed; one of the tumours produced lymph-node metastases and one gave rise to liver metastases [7]. Although cell lines cannot be considered representative of the diversity encountered in a primary tumour, these results indicate that the differences in growth and metastatic ability observed with cell lines are to be expected also when studying fresh tumour tissue in an orthotopic model.

An interesting possibility with the model here used is that implanted tumours may be resected at an appropriate time, mimicking the post-resection phase of colon cancer in man. Since the caecum is long and highly mobile in rodents, an established tumour can easily and safely be resected, provided that it has not grown too large. In a study with orthotopic xenografts, resections of 'primary' caecal tumours 10 days post-implantation were shown to prevent the formation of liver metastases [11]. Although only one of the tested cell lines in our study produced liver metastases, it is conceivable that with resection of the primary tumours, a different metastatic pattern might have been seen, as has been observed in other experimental tumour systems [12]. Such models could also provide a relevant system for studying micrometastases and efficacy of anticancer drugs in the adjuvant setting.

In summary, with a large panel of 12 colorectal cancer cell lines studied in an orthotopic model we found widely different patterns of growth and metastatic spread. Only with SW620 cells were liver metastases observed, whereas many of the cell lines metastasised to mesenteric lymph nodes at high frequencies. Although some variation in results are to be expected between different laboratories using different implantation techniques and strains of mice, we suggest that the results reported here may be of help for other investigators choosing cell lines for studies in orthotopic colorectal cancer models.

## 5. Conflict of interest statement

There are no financial or other interests with regard to the submitted manuscript that might be construed as a conflict of interest.

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