Jesse van Kuppeveld

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Supervisor: prof. dr. R. Coppes

Tumor-derived organoids improve colorectal cancer treatment

Abstract

Colorectal cancer is a deadly disease that is still difficult to treat. Conventional models do not represent the cancer well and treatment response is hard to predict. Recently, research has shown that intestinal stem cells can be maintained indefinitely in culture. This technique made it possible to create patient derived tumor organoids that reflect the disease more accurately than historical models do. These organoids copy key features of the parent tumor and can be used as a new model for colorectal cancer, with other possibilities like measuring patient response to drug treatment. Use of this model can make colorectal cancer treatment more efficient, reducing costs and leading to less patient toxicity. Applications of tumoroids as drug screening models will aid drug development as well, however, more research is needed to solidify their predictive value and culturing methods need to be standardized to standardize clinical use.

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Introduction

Colorectal cancer (CRC) is one of the top 3 most diagnosed cancers in men and women. An estimated 53.200 people will die from CRC in the USA this year, and the 5-year survival rate for colorectal cancer is 64%. About 95% of colorectal cancer cases develop sporadically, and the other 5% have an underlying genetic condition. Risk factors for developing colorectal cancer include age, gender, race, Inflammatory Bowel Disease (IBD), genetic conditions regarding DNA repair mechanisms, physical activity, nutrition and smoking (Colorectal Cancer – Statistics, 2020). Current treatment of colorectal cancer exists mostly of treatment with 5-fluorouracil (5FU) chemotherapy for metastatic cancer and radiation (RT) for muscle invasive and node-positive cancers (Janakiraman et al., 2020). Approximately 20% of the patients have a pathologically complete response to this treatment. To others, remaining tumor cells after treatment with 5FU/RT contribute to disease recurrence, and 40% of the patients undergoing this treatment will not benefit from it but will still be affected by the 5FU/RT toxicity (Janakiraman et al., 2020). When curative treatment is not possible, 5-year survival rate is 12,5%. It is believed that the primary for treatment failure is acquired resistance in the tumor, occurring in 90% of patients with metastatic cancer (Hammond et al., 2016).

Heterogeneity of the tumors results in different treatment responses for individual patients and thought to be one of the major reasons for disease reoccurrence. Tools to predict patient response currently do not exist and treatment has essentially stayed the same for more than a decade. Multiple drugs showing effectiveness in cancer models failed to do so in clinical trials, implicating that cancer models do not translate well into clinical practice (Ji et al., 2020). Current challenges in CRC treatment are chemotherapeutic resistance and the inability to predict treatment, and while progress is being made in cytotoxic and targeted therapy CRC treatment is far from perfect. Models used in CRC research and treatment do not accurately reflect the disease, hindering progression.

Current cancer models used are originally from primary tumors and mimic tumor development in the form of malignant cell lines and xenografts in immunodeficient mice. Cancer cell lines are cultured in 2D culture conditions and are maintained for many generations, potentially losing the genetic heterogeneity typical of colorectal cancer, rectal cancer cell lines are rare, and it is impossible to determine whether the material was pretreated and from what location it was derived. Patient derived xenograft models obtained from transplanting allow in vivo evaluation in contrast to cell lines but are costly to produce and have long establishment times (Ji et al., 2020).

Since current models still lack the detailed features of the actual disease in vitro, a new model is needed that can faithfully mimic tumors and is easy to establish. Recent developments in stem cell research enabled culturing of both healthy and cancerous epithelial stem cells in vitro. These 3D patient derived tumor organoids (tumoroids) resemble the parent tumors more closely, and have shorter establishment times, allowing for screening uses in the clinic.

Identifying a screening platform to predict patient response will prevent overtreatment and adverse effects on patient response and reduce treatment costs. Testing for all markers that influence drug response is unpractical and single mutations cannot accurately predict response to treatment. Anti-EGFR therapy decisions are largely dependent on KRAS mutation but only an estimated 35% of mutations have a response, highlighting the need for more accurate ways to predict treatment response (Linnekamp et al., 2015).
The goal of this thesis is to assess the viability of tumoroids as a drug screening platform both preclinical and clinical, and to identify areas where this technology might be improved.

Colorectal cancer progression

Colorectal cancer is a heterogenous disease, and as every cancer, develops after a series of genetic changes that causes the cell to lose control of proliferation. Colorectal cancer carcinogenesis is thought to start with mutations in the Wnt pathway and loss of APC, followed by mutations in MAPK, TGF-B and PI3K pathways (Fearon et al., 2010; Lau et al., 2020). The development of colorectal cancer requires several mutations to occur, and a large portion of the most recurrent mutated genes can be assigned to five signaling pathways: Wnt, PI3K, TGF, RAS/MAPK and TP53 (Fujii et al., 2016). Pathways that control cellular proliferation are thought to drive CRC carcinogenesis. While in CRC 3-6 driver pathway mutations can be found, most (>90%) of colorectal cancers contain less than five driver pathway mutations. Some tumors contain no alterations or only a single alteration, in these varieties, epigenetic changes or chromosomal disruptions might be responsible for the malignant transformation (Matano et al., 2015).

Standard investigation of CRC pathogenesis is based on analyzing the stage of the tumor, where the tissue transitions from healthy epithelium to an eventual carcinoma through mutations in the APC, K-RAS, DCC and TP53 genes (DeHaan et al., 2020). A large number of CRCs develop following two morphological pathways: the adenoma-carcinoma sequence or conventional pathway accounts for 60% of the cases, and the serrated neoplasia pathway that can be found in 35% of the cases. Lynch syndrome, a genetic condition characterized by mutations in mismatch repair genes, accounts for the other 5% of CRC cases. Improved understanding of the (epi)genomic landscaped would improve CRC classification. The adenoma-carcinoma sequence model, which states that inactivation of the APC gene creates a premalignant precursor lesion. After this first mutation other mutations occur in the KRAS, SMAD4 and TP53 genes, these mutated driver pathways further aid in shaping the lesion into a malignant tumor, making it possible for the tumor cells to survive beyond their ‘normal’ environment and metastasize in other locations of the body (Matano et al., 2015). The other model, the serrated neoplasia pathway, starts with a mutation in BRAF, activating the MAPK pathway. Hypermethylation of promotors takes place which inactivates tumor suppressor genes (Lau et al., 2020). Using tumoroids as a new model to study CRC with the ability to induce mutations via CRISPR-Cas9 can increase our understanding of CRC carcinogenesis (Matano et al., 2015). Leading to new therapeutic targets and better identification of tumor stages in the patient.

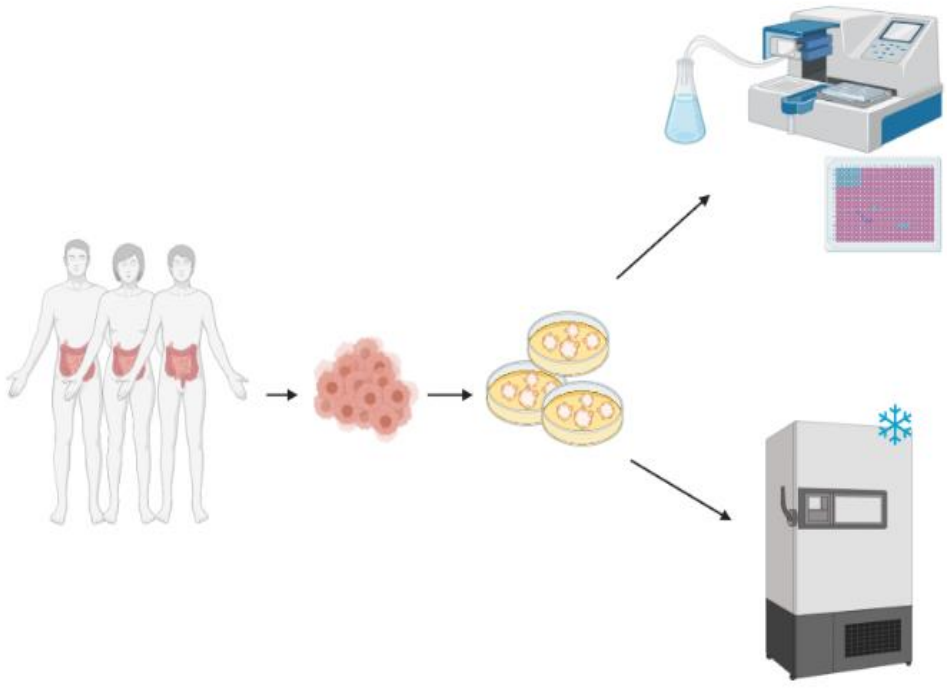
Historical cancer models

The current models used in CRC research, animal models, patient xenografts and cancer cell lines have aided in cancer research but have shortcomings in personalized medicine use. Cancer cell lines come from primary tumors and are grown in a 2-dimensional culture. This model is commonly used since it is cheap and easily handled, but it has its limitations. Cell lines lack the cellular heterogeneity tumors have, do not have matched healthy controls cell lines undergo genetic changes from long-time culturing (DeHaan et al., 2020; Xie et al., 2019; Lasabova et al., 2019).
The limitations of patient derived xenograft models include high cost, animal use and limited efficiency, and cannot accurately imitate the tumor microenvironment. Long establishment times and complex logistics for for high-throughput analysis disqualify their use in personalized medicine (Xie et al., 2019, Li et al., 2020). Murine animal models were difficult to fully use to model CRC since the tumors tend to form in the small intestine, however, newer methods try to circumvent this by transplanting a tumoroid in the colon (Clevers et al., 2019).
Patient derived xenografts are created by transplanting patient tumor cells in the intestine of an immunodeficient mouse. An advantage of this model is that implantation of the tumor better imitates the tumor environment than cell lines. Nontheless, one of the drawbacks of using xenografts is that the tumor microenvironment, which plays a big role in tumor progress, changes from human to murine (Yuki et al., 2020). Most experiments regarding CRC are done using animal cancer models, cancer cell lines and patient xenografts (DeHaan et al., 2020).

Organoids as a new ex vivo model

Organoids from intestinal stem cells were first described by Sato et al. (2009), when this group was able to grow epithelial cells from LGR5+ intestinal stem cells, forming crypt-villus structures in vitro using a newly developed culture system (Sato et al. 2009, 2011). Single stem cells were able to form a colony and differentiate into small structures resembling the crypt-like structures in the intestine, essentially forming a miniature organ that could be grown in vitro (Sato et al., 2009). The culture system they used is constructed using a lamilin rich extracellular matrix called Matrigel, to form a 3D structure, with the addition of Wnt3a, R-spondin 1, Noggin and EGF, and inhibitors of p38 MAP kinase and TGF-5b. Cancer cells usually do not need Wnt or R-spondin factors to be maintained in culture (Sato et al., 2009 & 2011; Ganesh et al., 2019).

Organoid technology has the potential to be used in a vast amount of applications, from a human derived model replacing animal models in trails, to potential tissue or organ regeneration. In oncology, tumor organoids (tumoroids) can potentially be used for personalized medicine and new accurate models for drug development. Investigations in these applications have already been done and will be mentioned in this thesis. Only recently, studies have shown that organoids derived from patient tumors and metastases can be grown with success (Ashley et al., 2014; Weeber et al., 2015; Van de Wetering et al., 2016), and tumoroids have since been used in studies to predict patient drug response (Pasch et al., 2019). Large biobanks, collections of harvested tumor or healthy (intestinal) cells from patients can also be created and expanded for drug screening or frozen for later use (Van de Wetering et al., 2015).



[1]

Carcinogenesis research can be performed using tumoroids as models, and mutations to investigate genetic changes can be induced using CRISPR-Cas9 (Drost et al., 2015; Matano et al., 2015; Fumagalli et al., 2017).
There is currently no consensus on the definition of an organoid. Therefore, in this article we follow

[1] Biobanking (Van de Wetering et al., 2015)

the definition of a 3D matrix embedded culture of continuously proliferative primary epithelial cells, are continuously proliferative in a Wnt-signaling and mitogen dependent manner (Clevers et al., 2019).

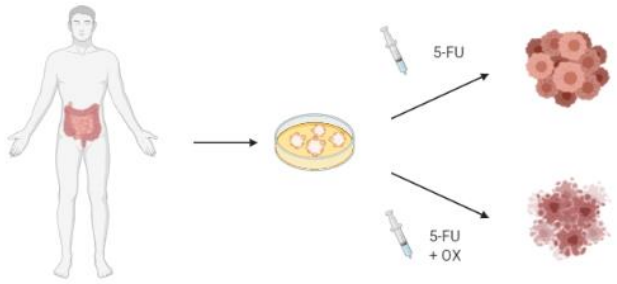
Patient derived CRC tumoroids retain histological and phenotypical features of the parent tumor, including secondary architecture, nuclear pleomorphism, nuclear to cytoplasmic ratio, presence of prominent nucleoli and mitotic rate, and the crypt-like structures characteristic of the intestine (Pasch et al., 2019). Expression patterns of the parent tumor remain largely the same after culturing (Ashley et al., 2009; Weeber et al., 2015; Pasch et al., 2019; Vlachogiannis et al., 2018). Tumoroids maintain the genetic mutations found in the parent tumor (Weeber et al., 2015). The retention of characteristics of the parent tumor make tumoroids an excellent CRC model, overcoming obstacles found in other cancer models, and allowing for personalized screening models. One of the advantages of using a patient derived organoid is that a healthy control can be made. As seen in tumoroids originated from other tissues, RC tumoroids also retained specific features found in the patient tumor. Glandular features, mucin production, nuclear stratification and other differentiation features were retained (Ganesh et al., 2019). Another study analyzed the transcriptomes of single cells derived from tumoroids and found that subpopulations could be identified with different sensitivities to chemotherapeutics commonly used in CRC treatment (Chen et al., 2018), suggesting that the heterogeneity in tumors is recapitulated in tumoroids. Other studies have also indicated that indeed cellular heterogeneity is maintained after culturing (Pasch et al., 2019).
Conservation of the original histological, genetic and phenotypical features by tumoroids could make them useful for the analysis of new therapeutics and new ways to tailor treatment plans to the needs of the patient.

Applications of tumoroids in colorectal cancer treatment

Tumoroids have the potential to be used for personalized treatment, showing the ability to mimic the tumor of the patient ex vivo. This could allow the tumoroid to be used for drug screening and set up a treatment plan based on the sensitivities and resistances of the tumoroid.

Tumoroids can be made from biopts taken during a standard endoscopy (Ganesh et all., 2019), and can be grown in a short amount of time (Pasch et al., 2019). The success rate of tumoroid establishment can be as high as 90% if enough material has been recovered (Van de Wetering et al., 2015), allowing for quick and easy tumoroid establishment in the clinic. An assay to create a patient derived tumoroid and screen it for irinotecan sensitivity could be set up within two weeks (Ooft et al., 2019). It has been shown that tumoroids can be grown with a high success rate if enough cells are used to establish the culture. In a study by Bing-Ying et al. (2016) they used four different densities of 500/1000/2000/4000 per well, with just 2000/4000 densities showing robust growth after 25 days. That tumoroids can be grown with a high success rate if enough cells have been harvested has also been reported in other studies (Ooft et al., 2019; Pasch et al., 2019). For tumoroids to be used in the clinic it is needed that the establishment of cultures is efficient and can happen within the timeframe to make a treatment decision.

An example of possible clinical use was shown by the team of Pasch et al. (2019). A tumoroid was created from the liver metastases of a patient with refractory metastatic CRC to be used to predict if retreatment with FOLFOX, a chemotherapeutic regimen containing 5-FU, leucovorin and oxaliplatin, would be beneficial. The tumoroid was resistant to 5-FU alone, but reduced in size after being given the combination of 5-FU and oxaliplatin. The patient was treated with FOLFOX based on this, resulting in a reduction of tumor markers and tumor diameter within two months after the start of the treatment. The treatment would continue to give this response for over a year. A clear example of using patient derived tumoroids to predict treatment response, this application in the clinic can aid in the treatment decisions for CRC patients, resulting in more efficient treatments and hereby reducing treatment costs and patient toxicity.



[2]

Rectal cancer (RC) tumoroids display different responses to radiation and chemotherapeutics. The team of Ganesh (2019) used RC tumoroids engrafted in mice to screen for drug sensitivity, instead of testing the tumoroids in culture. The mice were engrafted with a tumoroids derived from a more aggressive tumor or a less aggressive tumor and treated with 5-FU. The response of the mice matched the patient response in the clinic. Tumoroids that shrunk a small amount after being irradiated were derived from patients that did not respond well to radiotherapy, while tumoroids that were more sensitive to radiation and shrunk 50% or more were derived from patients that responded well to

[2] Tumoroid creation and screening (Pasch et al., 2019)

radiotherapy. 16 out of 17 patients with radiosensitive tumoroids had a good response to chemoradiation (Yao et al., 2020; Ganesh et al., 2019).

Multiple studies have been carried out in which they compared patient derived tumoroid treatment response to patient response. Several finding that for some therapeutics, an accurate prediction on patient response can be made (Ooft et al., 2019; Ganesh et al., 2019; Yao et al., 2020). Studies have compared tumoroid response and patient response to multiple chemotherapeutics and radiotherapy, in which they found that in most cases tumoroid response was similar to the patient response. Tumoroid response was 84% accurate for 5-FU sensitive tumoroids and 71% for 5-FU resistent tumoroids (Yao et al., 2020). This should be accurate enough to be able to use in the clinic to better tailor patient treatment.

The team of Yao et al. (2020) created 80 patient derived organoids and treated them with chemoradiation in a co-clinical trial to test if advanced RC tumoroids reliably can predict patient response. They found that 85% of the tumoroid sensitivities matched the patient response, meaning that if the tumoroid was sensitive to one of the three therapies given (5-FU, CPT-11, or radiation) the patient had a good response as well. If the tumoroid responded poorly to all of the treatments, then the patient also had a poor response. This demonstrated that tumoroid sensitivity and resistance can be a good indicator of patient response. Overall, the large amount of tumoroids used and the high percentage of sensitivity matches indicates that tumoroid responses can be used to make treatment decisions (Yao et al., 2020). Ooft et al. (2019) have demonstrated that CRC tumoroids can be used to predict patient response to irinotecan, by using a single concentration of the active metabolite at which the therapeutic effect is largest. The assay they used contained about 5000 cells, and could be grown and screened in 2 weeks, enough time to use clinically. However, a prediction could not be made to 5-FU + oxaloplatin treatment. This is thought to be because the mechanisms of action of chemotherapy are still quite unknown, and tumoroids for now still lack important parts of the tumor microenvironment like immune cells and the stroma (Ooft et al., 2019). Improving the tumor microenvironment and hereby hopefully improving the sensitivity of the tumoroids would bring this application closer tom clinical use.

Besides using tumoroids as tools to personalize patient treatment, large amounts of different tumoroids can be bio banked and be used to detect drug-genotype associations and high-throughput drug screens. Van de Wetering et al. (2015) created tumoroids and healthy organoids from the primary tumors of 20 CRC patients. These were used to screen compounds in a high throughput platform, analyzing 83 different compounds including drugs currently used in the clinic (n=25), chemotherapeutics (n=10) and drugs under investigation for clinical use or previously investigated (n=29). The high-throughput screening already showed potential for tumoroid use of biomarker identification. This study used 22 organoids in a first-time example of a biobank for such purposes, but a larger study using more organoids and including rarer variants of CRC would allow more biomarkers to be found, with better proof of their use. Creating CRC tumoroid biobanks allows the detection of relationships between tumor genotypes and drug sensitivity, paving the way for optimizing treatment plans based on tumor genotypes.

The application of tumoroids as a drug screening tool would be very valuable, since the percentage of cancer targeting drugs in development that end up being used in clinical treatments is very low (Hutchinson et al., 2011). Tumoroids can be used to assess the efficacy and toxicity of potential new compounds quickly before entering clinical trials, avoiding the costs accompanied by the lengthy trails it takes to develop new drugs. Therefore, it is important that the tumoroids faithfully reflect the condition in the patient.

Room for tumoroid improvement

Tumor organoids have been shown to be a promising tool in the treatment of CRC, both as a sort of patient avatar, or as a new screening model for drug development and carcinogenesis model. One of the current limitations is that most organoid/tumoroid models used in the experiments were cultured in submerged Matrigel and consist solely of epithelial cells (Van de Wetering et al., 2015; Ooft et al., 2019; Bing-Ying et al., 2016; Pasch et al., 2019; Yao et al., 2020; Ganesh et al., 2019) This does not fully represent the tumor in the patient, since tumors do not only consist of epithelial cells, but also of mesenchymal cells, immune cells and neural cells. Yuki et al. (2020) showed that immune cells influence carcinogenesis. Addition of CD4+ T cells from a murine breast cancer model, with and without Tumor associated macrophages to mouse mammary epithelial cell organoids showed that helper T cells, expressing CD4, increased organoid disruption and invasive behavior. Tumor associated macrophages activate epidermal growth factor signaling enabling metastasis. On the opposite, loss of CD4+ cells in the murine model reduced the metastatic potential of the tumor (DeNardo et al., 2009; Yuki et al., 2020; Lau et al., 2020).

New methods have been developed to be able to incorporate more cell types. Neal et al. (2018) used an Air-liquid interface (ALI) with Matrigel to be able to culture mesenchymal and epithelial cells together. This ALI preserves the genetic landscape of the original tumor as well as the cellular composition and architecture of the tumor microenvironment, as well as the tumor parenchyma and stroma with fibroblasts and immune cell (Yuki et al., 2020). Other methods can be used to include these cells in organoid cultures. Immune cells can be co-cultured with tumoroids to investigate interactions between the immune system and cancer progression (Neal et al., 2018). The addition of these cell types might lead to the discovery of new drug targets. Investigations into predictive factors for anti-angiogenic treatments have had little results, and some studies suggest a correlation between immune system and angiogenesis (Pedrosa et al., 2019).

A limitation of organoid use in laboratories is that the cultures being used all differ in composition leading to experimental variation in studies if there will not be an affordable standard culture for organoid development (Lau et al., 2020). A better-defined medium should be developed that removes the changing composition of Matrigel as an experimental factor.
Analysis of the tumors that failed to be cultured show the tumors depend on inflammation factors and pathogen exposure (Li et al., 2020). Tumoroid cultures cannot be established from all types of CRC (Li et al., 2020). Another factor in CR carcinogenesis are microorganisms found in the gut. Several species found in the human intestine are able to promote cancer progression and metastasis by influencing the immune system and through metabolites (Lau et al., 2020), incorporation of these bacteria or their metabolites can open up possible new therapeutic targets.

Tumors classified as MSI, BRAF-mutated, poorly differentiated or mucinous type were hard to grow in culture (Li et al., 2020). 10 to 20% of CRC cases are the mucinous type, and patients with this type of CRC receive the standard treatment of CRC. However, their response to the treatment is poor and some studies indicated their progression-free survival and overall survival is lower than non-mucinous types (Luo et al., 2019). Incorporating different cell types into the tumoroid culture can uncover new targets for drug development, unravel new mechanisms in CRC progression and aid in the establishment of tumoroid cultures.

Conclusion

Tumoroid models are promising models with different applications in cancer research. Studies using tumoroids and organoids derived from healthy tissue show results that indicate possible clinical uses, like measuring response to chemoradiation, chemotherapeutics and different drug combinations. Analysis of tumoroids show that they readily recapitulate key features of parent tumors in the patient and faithfully reflect the genetic landscape of the tumor. However, there are different reasons why the technique has not been widely implemented. Sample sizes are still too small to make an accurate prediction and, while several studies had reasonable sample sizes, larger studies are still needed. Other hindrances are that organoid technology is relatively new, the term has not been defined clearly yet and the culture media used in most studies differ in composition, making it harder to compare studies. Furthermore, culture establishment remains costly.
Organoid applications can be expanded by adding different cell types which make the microenvironment more similar to the tumor microenvironment in the human body. This may change in the future as these applications of organoids in cancer research are continuously investigated by different groups, but for now more research is needed before patient derived tumoroids can become standard practice in the treatment of CRC.

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