Unique Characteristics of Serrated Colorectal Cancer and Potential Avenues for Treatment

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Serrated colorectal cancer (CRC) is a subtype of CRC, characterized by tumors with a "serrated" or "saw-like" morphology under the microscope. Serrated CRC can be induced via multiple different pathways. One key initiating event is the mutation of the BRAF gene, which stimulates the mitogen-activated protein kinase (MAPK) pathway. However, the BRAF mutation alone is insufficient to cause tumorigenesis and may be succeeded by the transforming growth factor beta (TGF-β) pathway or Suppressor of Mothers Against Decapentaplegic (SMAD)-independent pathways. On the other hand, atypical protein kinase c (aPKC) deficiency can also lead to serrated tumors that quickly become invasive. In both cases, the amount of programmed death-ligand 1 (PD-L1), an immune checkpoint protein, increases dramatically, preventing T-cells from killing tumor cells. Therefore, the potential of anti-PD-L1 therapy as a novel treatment option was investigated. Since anti-PD-L1 therapy is only effective in early, less-advanced cancers, the possibility of TGF-β specific inhibitors as a treatment was also considered.

Keywords: colorectal cancer (CRC), BRAF, transforming growth factor (TGF)-β, atypical protein kinase c (aPKC), mitogen-activated protein kinase (MAPK), programmed cell-death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1)

Introduction

CRC is a type of cancer that is caused by mutations and cellular pathways that eventually lead to tumorigenesis, a state where normal cells are transformed into cancer cells[1]. Serrated CRC is a special, rarer subtype of CRC that is distinguished by its serrated or "sawtooth" morphology under the microscope[1]. Serrated CRC, although less common than typical CRC, is harder to detect and leads to worse patient outcomes[2]. The BRAF mutation increases the chance of death by 43% after multivariable adjustment[3]. Most colorectal tumors are formed through mutations in the WNT pathway, a signaling cell pathway that is involved in regulating cell growth, differentiation, and movement[4].

However, a smaller percentage of around 5-10% of CRCs are believed to be formed through the MAPK pathway, a pathway that regulates cell proliferation, differentiation, and mutations in the BRAF gene; this pathway leads to the development of serrated tumors[1]. However, the BRAF mutation is not the only pathway that can lead to serrated CRC. A mutation in the KRAS gene (typically codon 12/13), results in the activation of the MAPK signaling cascade, although it occurs in only 5% of serrated CRCs[5]. aPKC-deficiency, where there is reduced activity of atypical protein kinase C enzymes, is also a possible cause, which leads to aggressive serrated CRCs[5].

Serrated lesions (abnormal areas of tissue with abnormal growth) are classified by the World Health Organization into three subtypes: sessile serrated lesions (SSLs), traditional serrated adenomas (TSAs), and hyperplastic polyps (HPs), which can all transform into fatal cancers[6]. HPs are the most common type of serrated lesions, accounting for 60-75% of all serrated lesions[7]. They are typically small (<5 mm), exhibit a sessile (flat) morphology, and are found on the left side of the colon7. SSLs, which make up around 20-25% of serrated lesions, are frequently found on the right side of the colon, are typically 5-10 mm in size, and have a sessile (flat) morphology[7]. TSAs are the least common, accounting for less than 1% of serrated lesions, and can exhibit either a sessile (flat) or pedunculated (stalk-like) morphology[7]. These statistics are summarized in Table 1.

BRAF-driven serrated CRC is also strongly associated with extensive CpG Island Methylation Phenotype (CIMP) and microsatellite instability (MSI), which separates them from typical CRC[7]. CIMP refers to DNA modifications where methyl groups are added to cytosine bases within regions of the DNA (known as CpG islands)[8]. Generally, CIMP plays an important role in regulating tissue-gene expression.

However, in the case of serrated CRC, CpG islands are hypermethylated, affecting gene expression and potentially silencing tumor suppressors[8]. On the other hand, MSI happens when the repeated DNA bases in a microsatellite (short, repeated sequences of DNA) are different from the original sequence, potentially leading to mutations[9].

Features	Hyperplastic polyps (HPs)	Sessile serrated lesions (SSLs)	Traditional serrated adenomas (TSAs)
Percentage of all serrated lesions	60-75%	20-25%	<1%
Morphology	Sessile (flat)	Sessile (flat)	Sessile (flat) or pedunculated (stalk-like)
Size	<5 mm	5-10 mm	>5 mm
Location	Distal (left side of colon)	Proximal (right side of colon)	Distal (left side of colon)

Table 1 Three subtypes of serrated lesions and their characteristics

Research Questions and Methods

This report aims to summarize recent studies examining the unique characteristics of serrated CRC, compare different pathways for its initiation, and discuss potential treatments as well as ongoing and future initiatives for research. A few research questions are listed below. First, what are the characteristics of serrated CRC that distinguish it from typical CRC? Second, what are the pathways of initiation for serrated CRC? How do these pathways differ from the initiation of typical CRC? Third, what are the potential treatment options for serrated CRC? What should future studies focus on?

To gain a basic understanding of the complexities of serrated CRC, recently published review articles and empirical articles were analyzed, summarized, and compared. A large compilation of sources was listed in the references of other review articles, which was employed to focus on specific aspects of serrated CRC more aligned with ongoing research. Research was primarily concentrated on the causes of serrated CRCs, including aPKC loss and the TGF-β pathways. Forms of treatment for serrated CRC were also a major topic of investigation. Additionally, articles that placed particular emphasis on murine models and the $BRAF^{V600E}$ mutation were investigated. These articles were compiled from full-text databases like PubMed, Elsevier, Cell Press, Frontiers, Europe PMC, and Oxford Academic, all of which contain exclusively peer-reviewed articles. Additional journals such as the New England Journal of Medicine, British Journal of Cancer, EXCLI Journal, Histopathology Journal, and EMBO Molecular Medicine Journal were utilized for their vetted peer-reviewed content.

To ensure relevance to the field, every article used in this paper had its citations by other articles tracked as indicated by PubMed citation tracking. In order to strike a balance between relevance and novelty, as of 10 September 2024, the articles used in this paper have a range of 0 to 2,408 citations by other articles, with an average of 247 citations each. In order to gauge the accuracy of the review articles, the information presented in the article was cross-referenced across multiple articles with similar topics. For original research papers, only peer-reviewed articles were considered. In addition, all methodologies were checked for clear, replicable instructions; any research article without clear methodologies was disregarded. Also, research articles were checked for the inclusion of effective control groups.

Significance of Serrated CRC Research

Research into CRC, in general, is significant in providing insights into one of the most widespread forms of cancer. CRCs were the third most frequent form of cancer in both males and females in 2023 [10]. Moreover, serrated CRCs result in a much higher risk of serious illness compared to normal forms of CRC5. Research focusing on the pathways that induce serrated CRC provides insights into possible causes of the disease. For example, investigating the MAPK pathway is critical to understanding the significance of the $BRAF^{V600E}$ mutation in serrated CRCs. However, there may also be other pathways at work, including the TGF-β pathway, which must not be overlooked [11]. aPKC deficiency is also investigated as a cause for serrated CRC. Exploring possible treatments, such as TGF-β specific inhibitors and anti-PD-L1 therapy, will aid in relieving the effects of the disease on patients.

Serrated Tumor Initiation

Events Beyond the BRAF Mutation Initiation

Although mutations in the BRAF gene (such as $BRAF^{V600E}$) may contribute to serrated CRC, they are insufficient to drive tumorigenesis alone, as demonstrated by tests using the Villin-CreERT2 driver, which was not previously used for studies of $BRAF^{V600E}$ -driven intestinal tumorigenesis [1]. In this study, the authors conditionally activated either one or two alleles of the BRAF oncogene within the adult intestinal epithelium using the conditional Villin-CreERT2 driver and observed any changes post-tamofixen-induced activation [1]. Villin-Cre mice are a type of genetically engineered mouse models that express the Cre recombinase enzyme in the epithelial cells of both the villi and crypts of the small and large intestine [12]. The Cre recombinase enzyme is a biological tool that allows researchers to edit genes by targeting specific DNA recognition sites, which gives researchers precise control over gene expression in various contexts [13]. Previous studies investigating colon tumorigenesis have found that the $BRAF^{V600E}$ oncogene is insufficient in promoting serrated CRC tumorigenesis in mouse model systems [14,15]. Although there is a lack of research showing mouse models' successful driving tumorigenesis in serrated CRC, there have been studies investigating tumorigenesis in regular CRC. For example, Azoxymethane (AOM) mouse models are a type of

carcinogen-induced model, where mice receive intraperitoneal injections of AOM that induce tumorigenesis and lead to the development of sporadic CRC[16]. In addition, genetically engineered mouse models such as the $Apc^{Min/+}$ mouse model are effective in studying and replicating colon tumorigenesis[16].

The mutated BRAF oncogene causes continual activation of the MAPK pathway, which plays an important role in cell proliferation. Unexpectedly, when tested alone, the $BRAF^{V600E}$ mutation caused senescence—the process in which cells lose the ability to divide and grow—leading to increased cell differentiation; at the same time, a reduced number of stem cells were observed[7]. As most colorectal cancers originate from stem cells, which only require one mutation in the WNT/β-catenin pathway, the loss of stemness from the $BRAF^{V600E}$ mutation would reduce tumor incidence; therefore, the $BRAF^{V600E}$ mutation did not effectively cause serrated tumors by itself in mouse models[1]. However, upon manual reduction of cell differentiation-promoting genes such as SMAD4 and CDX2, stem cell numbers returned to normal and the mouse models were able to form serrated tumors, showing an inverse relationship between cell differentiation and vulnerability to BRAF-driven serrated CRC[1]. For example, mice with both the $BRAF^{V600E}$ mutation and the SMAD4 mutation displayed dysplastic lesions after one month and large tumors after two to three months[1]. In one study, BRAF-mutated tumors exhibited an increased expression of immune checkpoint proteins programmed cell-death protein 1 (PD-1)—found on T-cells—and PD-L1—found on cancerous cells—that inhibit T-cell activation via PD-1, suggesting a more immunosuppressive environment in BRAF-mutated tumors[17,18]. Although the specific events beyond the initial BRAF mutation are not well known, there are a few possibilities.

One possibility is the TGF-β signaling pathway, which is involved in cell proliferation, differentiation, migration, and apoptosis; it is one of the most altered cellular signaling pathways found in human cancers[11]. Mutations in the TGF-β signaling pathway occur in at least 30% of BRAF-mutated CRC. In addition, alterations of different components of the TGF-β signaling pathway have different effects on the microsatellite status of tumors[7]. Interestingly, the TGF-β signaling pathway plays a paradoxical role in the progression of colorectal cancer. While TGF-β inhibits normal colonic epithelial cells' proliferation and acts as a tumor suppressor, it also promotes the survival, invasion, and metastasis of colorectal cancer cells, thereby also acting as an oncogene[11]. In the normal intestinal epithelium, TFG-β acts as tumor suppressor, inhibiting cell proliferation and inducing apoptosis[11]. However, during the later stages of colorectal cancer formation, TGF-β acts as a tumor promoter and is usually highly expressed[11]. The mechanism by which TGF-β switches its inhibitory effect (to prevent cancer) into its growth-stimulatory effect (promoting cancer) is not well understood and is a part of ongoing research[11]. However, TGF-β has been shown to increase the production of mitogenic growth factors such as TGF-α, fibroblast growth factors, and epidermal growth factors. In addition, TGF-β can also activate SMAD-independent pathways, such as the MAPK, c-Jun N-terminal kinase, and phosphatidylinositol 3-kinase/Akt pathway[11]. Therefore, the TGF-β pathway may drive the proliferation of CRC cells in conjunction with these other pathways. In addition, many colorectal cancers are able to avoid the tumor-suppressor effects of TGF-β and are resistant to TGF-β-induced growth inhibition[11]. Given these conditions, it is likely that colorectal cancer cells are able to achieve resistance to the suppressive effects of TGF-β but remain responsive to its tumor-promoter effects through selective alterations of the pathway[11]. Additionally, the TGF-β pathway, although independent, is complementary to the PD-1/PD-L1 pathways[17].

However, a major clinical gap remains between the detection of serrated lesions and the development of therapies to treat them. Most CRCs, especially those with high MSI, respond poorly to chemotherapy[5]. Thus, anti-PD-L1 therapy and specific TGF-β inhibitors may be possible treatments for this subtype of cancer driven by BRAF mutations.

aKPC-deficiency

aPKC has two subtypes—PKCζ and PKCλ/L—which are encoded for by the PRKCZ and PRKCI genes respectively[19]. Specifically, PKCλ/L is a regulator of intestinal inflammation and plays a role in Paneth cell differentiation, which secrete antimicrobial peptides[20]. Knockout of PRKCI causes a lack of adequate Paneth cells, which impairs the intestinal epithelial barrier and causes inflammation. Contrary to the knockout of PRKCI, PRKCZ knockout downregulates the interferon response and leads to higher levels of immunosuppression[19].

Inactivation of both aPKCs leads to serrated hyperplasia, sessile serrated adenomas-polyps, dysplasia, and adenocarcinoma development without the need for additional mutations, unlike BRAF[19]. Additionally, PRKCZ and PRKCI knockout mice display microsatellite stable (MSS) and low CIMP phenotypes, are poorly differentiated and highly invasive, and resemble BRAF-mutant serrated tumors[5]. However, while BRAF-mutant mice are MSI, aPKC-deficient mice are MSS; this means that aPKC-deficient tumors cannot usually be treated with single-agent immunotherapy[19]. Furthermore, aPKC-deficient mice typically have poor prognoses, especially because of their highly immunosuppressive properties. These include the exclusion of cytotoxic CD8+ T-cells from interfering with the tumor and the increase of regulatory T-cells and myeloid-derived suppressor cells[5]. Although the proportion of CD8+ T-cells in these tumors is normal, there is a huge spike in PD-L1 expression[19].

Similar to MSS BRAF-driven serrated CRC, one potential treatment for aPKC-deficient tumors is the pairing of TGF-β specific inhibitors with anti-PD-L1 therapy. TGF-β specific inhibitors arrest progression to invasive cancer, which is crucial

because anti-PD-L1 is only effective in early, less-advanced cancers[19]. One study that used a combined treatment of TGF-β receptor inhibitor plus anti-PD-L1 checkpoint blockade showed synergistic curative activity[19].

Anti-PD-L1 Therapy/PD-1 Blockade

Recent research suggests that a promising avenue of treatment for serrated CRC could be observed in the checkpoint blockade of PD-L1 and PD-1. When PD-L1 and PD-1 interact, T-cell activity, and in turn, immune response to cancer, becomes diminished. Checkpoint blockade, in general, works by blocking this interaction, allowing the immune system to recognize and eliminate cancer cells more rapidly[21]. Serrated CRCs are associated with high MSI, correlating with higher PD-L1 expression. In addition, PD-L1 expression is increased when tumors have a BRAF mutation[22]. Due to the high amount of PD-L1 expression that is inherent in serrated CRCs based on its characteristics, anti-PD-L1 blockade seems especially useful for this form of CRC. In addition, one advantage of PD-L1 or PD-1 immune checkpoint blockade is its ability to aid cancer treatment and lessen the extent of possibly damaging adverse events, with skin toxicities being the most common side effect[21]. For patients with low PD-L1 expression in tumor cells, aspirin administration may also have some potential to further increase the effectiveness of PD-L1/PD-1 checkpoint blockade[22].

Monoclonal antibodies (mAbs) have been effective in treating solid tumors by blocking PD-1 or PD-L1 receptors, which restimulates the immune system[21]. A notable example of a monoclonal antibody is pembrolizumab, which has been approved by the FDA as both a first-line and second-line treatment targeting PD-1 on T-cells[21]. Other PD-1 inhibitors that have been FDA-approved include nivolumab and cemiplimab, and PD-L1 inhibitor antibodies that have been FDA-approved include atezolizumab, durvalumab, and avelumab[22]. However, there have been no clinical trials so far regarding the use of monoclonal antibodies applied to patients specifically with serrated CRC and not a more traditional form of the cancer. But since serrated CRC is strongly associated with MSI, and there have been positive responses when monoclonal antibodies were used to treat MSI forms of CRC, it stands to reason that investigating possible treatments about this characteristic would be effective in treating the majority of serrated CRC. For example, one clinical trial involving 74 patients with MSI in a two-year span, nivolumab was evaluated to be able to substantially improve long-term survival for CRC patients[23]. In addition, a 2025 trial involving 11 patients that were treated with pembrolizumab and XL888 (Hsp90 inhibitor) suggested immune activation, with Hsp 90 inhibition having the potential to be used with other monoclonal antibodies[24]. However, a study involving mouse models brought up the possibility that resistance to PD-1 blockade may encourage upregulation of other immune checkpoints mitigating the effects of monoclonal antibodies[25]. While this study exclusively focused on immune checkpoint blockade for lung cancer, it provides some potential limitations of PD-1 blockade treatment, which may or may not be applicable to serrated CRC[25]. As more clinical trials are conducted that specifically target serrated CRC, other possible resistance mechanisms to mAbs may be discovered that may apply to humans rather than to preclinical mouse models.

Combination therapy involving these mAbs has the potential to be effective and reduce the possibility of increasing resistance to other mAbs. Particularly, pembrolizumab combined with the cytotoxic T-lymphocyte associated protein 4 (CTLA-4) receptor inhibitor ipilimumab showed superior results compared to monotherapy with either treatment[21]. Before then, nivolumab has already been approved as a first-line therapy by the FDA and could also be used in combination therapy with ipilimumab[21,22,26]. It has also been shown that tumors located on different sides of the colon respond differently to different monotherapies, perhaps implicating that combination therapy would yield superior results[26]. However, the harmful adverse effects associated with combination therapy shouldn't be ignored. For example, the combination therapy involving nivolumab and ipilimumab has increased rates of grade 3 and 4 toxicities when compared to Nivolumab monotherapy[26].

TGF- β Specific Inhibitors

To aid the efficacy of anti-PD-L1 therapy, TGF-β specific inhibitors prevent the TGF-β pathway from progressing in BRAF-mutant and aPKC-deficient serrated CRC. This has many critical effects, most prominently the deduction in CD8+ T-cell exclusion from fibroblast interference[27]. A study done on mouse models showed that administering only anti-PD-L1 therapy or TGF-β specific inhibitors did not reduce tumor burden[27]. However, when combined, 70% of mice had a complete response and CD8+ T-cell infiltration increased significantly[27]. A phase I clinical trial of a bifunctional fusion protein inhibiting both TGF-β and PD-L1, bintrafusp alfa, also showed promising initial results across a variety of cancers, such as a complete response in a patient with cervical cancer. However, the sample size was small and the two patients with colorectal cancer showed no signs of improvement, so more research is needed[28].

Another study focused on inhibiting the TGF-β pathway in CRC by using Pirfenidone (PFD), a previously FDA-approved treatment for idiopathic pulmonary fibrosis. It has been used to decrease inflammation in various other cancers as well. PFD was found to significantly reduce TGF-β expression in both mouse and human CRC cell lines, in addition to reducing the growth and viability of mouse and human CRC cells by lowering survivin mRNA expression. PFD on its own induced apoptosis and increased tumor necrosis by increasing BAX expression in tumor tissue, though this was more prevalent in mouse cells.

When combined with chemotherapy, PFD drastically increased incidences of apoptosis in human cells[29].

Current Research

Research findings in 2024 have expanded upon the investigation of the effects of TGF-β signaling and the BRAF mutation. Recent research suggests that gastrointestinal stromal tumors have been affected the most by TGF-β signaling. In a study, stroma that is TGF-β activated was investigated to relate its presence to serrated CRCs. The study suggests how TGF-β-dependent stromal activation may occur early on in formation for serrated CRC10. However, TGF-β signaling throughout the lifetime of the tumor is a complicated topic, and further research is needed. Another 2024 study found distinct features of dangerous BRAF-mutated proximal colon adenocarcinomas[30]. Such characteristics include greater numbers of lymphatic invasions and number of lymph node metastases, as well as higher tumor budding[30].

In addition, Lymphocyte activation gene 3 protein (LAG3) was recently identified to be a possible third checkpoint for numerous cancers, including colorectal cancer[31]. Therapeutic treatments have also been a topic of current research. Another recent report analyzed TGF-β signaling and investigated possible therapeutic avenues. In addition to considering the possible anti-PD-L1 and TGF-β inhibitor combination therapy that is discussed in this report, other TGF-β inhibitors were also examined[32]. The report also considered combining TGF-β inhibition with other forms of therapy, such as radiotherapy[32]. Immune checkpoint blockade in combination with oxaliplatin-based chemotherapy was also conducted for three patients that have a subgroup of MSS CRC that is more resistant to checkpoint blockade showed remarkable results in eliminating the primary tumor and achieving complete response[33]. However, the recurrence of the tumor was much more malignant. In addition, another recent article explored the possibility of multi-pathway combination therapy to improve the effects of PD-L1 blockade for MSS CRC[34]. Furthermore, an investigative new drug known as benzosceptrin C shows potential in disrupting the interaction between PD-L1 and PD-1 and suppressing the growth of malignant tumors, especially when used in combination with a blockade of CTLA-4[35]. These recent findings show promising avenues for treatments that could be applied to treating patients.

Limitations

As a rule of thumb, this paper was limited by the extensiveness and criteria of the experimental trials. Many of the studies mentioned that a small control/experimental group or limited patient data restricted the validity of their findings. This applies especially to the adjuvant use of TGF-β inhibitors with PD-1 checkpoint blockade. Moving forward, both preclinical and clinical studies are needed to explore the full potential of PFD and other TGF-β inhibitors under different conditions.

One of the main limitations of this study is the variability of mouse models used to study serrated CRC. The commonly used Villin-Cre mouse model targets most cells that line the crypt-villus axis, which may limit understanding of the cell of origin for serrated CRC[7]. Additionally, not all mouse models behave the same. Some genetically engineered mouse models exhibit CIMP, MSI, and WNT signaling activation, while others do not[7]. The exact reason these inconsistencies exist remains unknown since they are part of ongoing research[7]. In addition, mouse models of BRAF activation have inconsistent evidence that BRAF causes senescence or stem cell differentiation during tumor progression1. However, some of these differences may be attributed to embryonic versus adult-onset activation of BRAF or varying levels of expression of mutant BRAF between studies[1].

Since each mouse model has its unique advantages and disadvantages, models should be chosen based on the expectations and aims of studies[36]. In general, since genetically engineered mouse models only provide evidence in the early stages of diseases, it is primarily advantageous for studies focusing on the initial phases of disease and select therapeutic studies such as chemoprevention studies seeking to prevent the onset of disease in the first place[36]. On the other hand, xenograft mouse models are better for studying carcinogenesis (the process by which normal cells are transformed into cancerous ones) and investigating the effects of therapeutics[36]. However, xenograft models are not good models to use for metastatic studies and studies investigating tumor-microenvironment interactions[36].

These limitations for each mouse model must be considered for the efficacy of each treatment method recommended. Regarding the efficacy of using both TGF-β specific inhibitors with anti-PD-L1 therapy, the study in mind crossed *Prkci*fl/fl and *Prkcz*fl/fl mice with Villin-cre mice, both genetically engineered mice, to get desired gene deletions[19]. The authors mention that when mice were treated with both galunisertib (a TGF-βR1-specific inhibitor) and anti-PD-L1, there was a reduction in the number, size, load, and aggressiveness of tumors[19]. However, the extent of this claim may be limited in the later stages of serrated CRC since the mouse models in the study did not reach metastasis[19]. While suppressing TGF-β signaling using galunisterib did slow the progression of CRC, the effectiveness of galunisterib during later stages of serrated CRC, especially in humans, is still unknown since genetically engineered mouse models only partially replicate the physiology and morphology of tumors[36].

Looking at the limitations of anti-PD-L1 Therapy/PD-1 blockade alone, one study using immunocompetent genetically engineered mouse models described that resistance to PD-1 blockade may encourage upregulation of other immune checkpoints mitigating the effects of monoclonal antibodies[37]. However, the

applicability of this claim is limited since the study used mouse models with lung cancer, which behave differently from genetically engineered mouse models of serrated CRC[37]. Additionally, while these genetically engineered mouse models are suitable for therapeutic studies in the early stages of cancer, the behavior and mechanism that causes the upregulation of immune checkpoints in the study may be different during metastasis and later stages of serrated CRC.

Regarding the effectiveness of using PFD to inhibit the TGF-β pathway, a few factors must be considered. First, the study in question utilized xenograft mouse models, with tumors injected subcutaneously into the mice. However, these models are immune system deficient, which limits the ability for researchers to evaluate the potential responses the immune system will have to PFD. Since the TFG-β pathway can act as an oncogene and suppress immune system functions, it may behave differently in mouse models with immunocompetent models. In addition, since xenograft models have low metastatic potential and TGF-β is involved in promoting metastasis, the study may overestimate the effectiveness of PFD at reducing tumor development, especially in the later stages of CRC.

Considering that the conclusions made about using various mAbs as anti-PD-L1/PD-1 blockade mainly came from various clinical trials, limitations regarding the surrounding clinical trials must be considered. For example, as stated previously, there have been no clinical trials as of now investigating the efficacy of mAbs being used for patients with serrated CRC. In addition, many of the clinical trials are limited by small sample size and potential demographic bias.

In addition, although anti-PD-L1 therapy/PD-1 blockade may serve as a potential form of immunotherapy, one article suggests that the opposing roles of molecular markers, KRAS and BRAF, may implicate immunotherapy[38]. According to the study, while mutated BRAF tumors displayed increased immune-cell infiltration compared to wild-type BRAF tumors, the opposite was seen for KRAS[38]. Specifically, the study found that when the number of cytotoxic T-cells increased, the proportion of mutated KRAS decreased. In contrast, the proportion of mutated BRAF increased[38]. However, it is important to know that this study also has limitations, with the authors mentioning a small sample size in the primary patient cohort and the possibility that the tumor tissue microarrays (TMAs) do not reflect the heterogeneous distribution of the infiltrating immune cells within the whole tumor[38]. Nevertheless, the immune system's response to the common molecular markers of serrated CRC may lead to predictive and prognostic tools for detection as well as treatment, but currently, there is limited understanding[38].

Moving Forward: Future Initiatives for Research

Many avenues for future research on serrated CRCs remain unexplored or require further study. One such avenue is developing new treatments to achieve complete response and increase progression-free survival (PFS) in MSS-serrated CRCs. One study shows complete response and a promising PFS of 20.7-35.0 months, as opposed to the median PFS of 4.0 months in the control group, by treating MSS serrated CRCs with immune checkpoint blockade and oxaliplatin-based chemotherapy[33]. Treating the recurrence of these cancers, which happened at previously tumor-free sites and sanctuary organs, has grounds for future research. However, due to the small sample size of 3 patients, more studies with bigger sample sizes are needed to confirm these results. Another study had one patient with MSS-serrated CRC achieve complete response and a PFS of $>$26 months, but this also requires validation with a bigger sample size[39]. In fact, there is only one targeted therapy approved for MSS-serrated CRCs[39]. More generally, most novel studies such as these, especially ones that serve as proof of concept, require validation with larger sample sizes.

Other potential paths for future research include identifying the reasons for the development of serrated lesions into malignant tumors. Serrated CRCs lose their serrated morphology as they become more invasive, so determining molecular biomarkers for serrated CRCs would be useful for tracking disease progression[7]. Furthermore, the exact mechanisms in which serrated CRCs turn from benign to malignant are poorly understood; the tumor microenvironment of different subtypes of serrated CRCs, especially if and how they change when progressing to malignancy, requires more research as well[7].

Conclusion

Overall, serrated CRC is a unique subtype of CRC due to the pathways that lead to tumorigenesis and the difficulty in treating it. Unlike most CRCs, which progress through the WNT pathway, serrated CRC can form via the MAPK pathway through either BRAF or KRAS mutations. Serrated CRC is also unique because it's strongly associated with CIMP and MSI. However, most CRCs, especially ones with MSI, respond poorly to chemotherapy. MSS-serrated CRCs also cannot be treated with immunotherapy. Therefore, TGF-β inhibition combined with anti-PD-L1 therapy could be a potential treatment for serrated CRC. However, the shortcomings and implications of this novel treatment are largely unexplored. The promise of novel therapy prospects, particularly therapy like PD-1 checkpoint blockade, is highly encouraged for further research.

Abbreviations

atypical protein kinase c: aPKC
colorectal cancer: CRC
cytotoxic T-lymphocyte associated protein 4: CTLA-4
pirfenidone: PFD

hyperplastic polyps: HPs
lymphocyte activation gene 3 protein: LAG3
microsatellite instability: MSI
microsatellite-stable: MSS
mitogen-activated protein kinase: MAPK
monoclonal antibodies: mAbs
programmed cell-death protein 1: PD-1
programmed death-ligand 1: PD-L1
progression-free survival: PFS
sessile serrated lesions: SSLs
suppressor of mothers against decapentaplegic: SMAD
traditional serrated adenomas: TSAs
transforming growth factor beta: TGF-β
tumor tissue microarrays: TMAs

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