The Potential Impact of Chromosomal Instability and Aneuploidy on cell-cell competition during Colorectal Cancer development

By

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Plain language summary

Colorectal cancer (CRC) is one of the most widespread and deadly cancers worldwide. When doctors discover a tumor in a CRC patient, tumors will be scanned and labeled as either microsatellite-stable (MSS) CRC or microsatellite-instable (MSI) CRC. In MSS CRC, the repair of DNA errors is still active, whereas in MSI CRC, this repair is non-functional. Importantly, MSS CRC tumors are also referred to as ‘’cold’ tumors, because they do not trigger a strong immune response. As MSS CRC makes up about 85% of all CRC cases, and cases are projected to rise among younger populations, it is crucial to research the molecular mechanisms underlying the development of MSS CRC.

The first DNA mutation observed in MSS colorectal cancer often occurs in a gene called *adenomatous polyposis coli* (APC). In healthy cells, APC helps in controlling how cells grow and divide. However, when this gene mutates, cells can start to grow uncontrollably. Over time, additional mutations start to build up in other genes that are responsible for controlling cell growth or repairing damaged DNA. Each of these changes can give the cells new advantages that help the tumor grow, eventually leading to cancer.

In addition to these mutations, another important piece in this puzzle involves intestinal stem cells (ISCs), which are responsible for constantly renewing the cells in the intestine. ISCs are located at the bottom of the intestine in small pit-like structures called crypts. Each crypt contains 5 – 16 ISCs that continuously balance either: (I) dividing into a new ISC or (II) being pushed up the crypt to turn into another type of cell to renew the intestinal cells. Importantly, these ISCs also compete with each other in a balanced way to take over space and resources in this crypt. For example, a slightly more competitive ISC can force another ISC out of the crypt to turn it into another cell type. This process is referred to as cell-cell competition. This process continues until the most competitive ISC takes over the crypt. However, when a mutation happens in one of these ISCs, like in the APC gene, these ISCs will have a competitive advantage. Essentially, they will dominate the crypt, which can result in the formation of small benign growths called adenomas.

In addition to these mutations and the concept of cell-cell competition, another important feature is something called “chromosomal instability” (CIN). CIN is a complex phenomenon that happens when cells make more mistakes as they divide. Normally, cells contain 46 chromosomes, however mistakes in cell division can lead to an abnormal number of chromosomes by either gaining or losing an extra chromosome, a condition known as aneuploidy. Aneuploidy then generally causes problems with growth, production of new proteins, and problems with metabolism. Although aneuploidy is generally stressful for cells, over 90% of MSS CRC tumors display aneuploidy in their cells. This raises the question of whether aneuploidy can play a role in cell-cell competition. In this review, I aimed to shine a light on this understudied topic.

In conclusion, I found that much is known about how specific mutations can contribute to cell-cell competition and influence the development of CRC. However, there is not much known yet about the role of aneuploidy on cell-cell competition. However, as technology advances and scientists learn new methods to model and study aneuploidy and cell-cell competition, it remains a matter of time before more is known about the molecular mechanisms underlying MSS CRC development.

Abstract

Colorectal cancer (CRC) is one of the major cancers resulting in death worldwide. Microsatellite stable (MSS) CRC, comprising approximately 85% of all cases, follows a progression model characterized by sequential mutations in key genes, including APC, KRAS, and TP53. These genetic mutations can have consequences on the progression from a healthy gut to a diseased gut, in which intestinal stem cell (ISC) dynamics and cell-cell competition play an important role. Importantly, MSS CRC progression is also closely linked to chromosomal instability (CIN) and aneuploidy, as aneuploidy is observed in 90% of MSS CRC tumors. The influence of CIN and aneuploidy on CRC initiation and progression remains an active area of research. This review explores the potential impact of CIN and aneuploidy on cell-cell competition of ISCs in MSS CRC development. In section I, I elaborate on CIN and aneuploidy in CRC development. Section 2 involves cell-cell competition in the healthy and diseased gut and the potential role of CIN and aneuploidy in this process. Section 3 explores how the influence of CIN and aneuploidy on cell-cell competition in the gut can be studied. With this review, I aim to shine a light on the understudied topic of CIN and aneuploidy on cell-cell competition in CRC development. I provide an overview of (I) CIN and aneuploidy in CRC development, (II) cell-cell competition in CRC development, and (III) methods to study aneuploidy in cell-cell competition

Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide, ranking third in cancer incidence and second in mortality. In 2020, over 1.9 million new CRC cases and approximately 930,000 deaths were recorded globally, highlighting the significant public health burden. With CRC incidence projected to rise particularly among younger populations, understanding the molecular mechanisms driving its development is crucial[2,3]. Approximately 85% of CRC cases are microsatellite stable (MSS), a type of CRC that does not involve mutations in mismatch repair genes.

The understanding of CRC development underwent a major shift in 1990 when Fearon and Vogelstein introduced the multistep genetic model of CRC progression. This model proposed that CRC arises, not from a single mutation, but through a series of specific genetic mutations that occur at different stages, driving the transformation from normal epithelium to malignancy. The process initiates with a mutation in the *adenomatous polyposis coli* (APC) gene, a tumor suppressor essential for controlling cell proliferation. Loss-of-function mutations in APC observed in 80% of MSS CRC cases, are typically the first mutations to occur. In later stages, gain-of-function mutations in *Kirsten rat sarcoma virus* (KRAS), and *phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha* (PIK3CA), as well as loss-of-function mutations in tumor-suppressing genes (TSG), such as *tumor-protein 53* (TP53) and *Mothers against decapentaplegic homolog 4* (SMAD4) are prevalent. It is the accumulation of these mutations, rather than their order, that drives the progression from benign adenomas to invasive carcinomas[4,5].

The progression from normal epithelium to invasive carcinomas is closely linked to the dynamics of intestinal stem cells (ISCs), which play a crucial role in the continuous regeneration of the intestinal lining. ISCs reside at the base of small glandular structures called crypts, where they maintain homeostasis through a process known as neutral competition. In this process, each ISC competes for space in the crypt, and those that remain at the crypt base retain their ability to self-renew[6]. Cells pushed upward differentiate into transit-amplifying (TA) cells, which proliferate and ultimately renew the intestinal lining[7]. Over time, one ISC clone will stochastically outcompete others, leading crypts to become monoclonal[8]. In the adenoma-carcinoma model, the initial APC mutation is believed to occur in ISCs giving rise to APC-mutant clones that contain a competitive advantage and gradually replace wild-type ISCs. As these APC^- mutant cells colonize the crypt, they give rise to the formation of adenomas and later oncogenic mutations drive the progression to invasive carcinomas[9,10].

Apart from mutations in oncogenes and TSGs, MSS CRC tumors also display chromosomal instability (CIN), i.e. an increased rate of chromosome mis-segregations during cell division. CIN can give rise to an abnormal number of chromosomes – or aneuploidy[11], and over 90% of MSS CRCs have aneuploid karyotypes. The influence of CIN and aneuploidy on CRC initiation and progression remains an active area of research, and recent studies in transgenic mice suggest that CIN can drive tumorigenesis in the mouse intestine[12]. It remains, however, unclear how CIN and aneuploidy influence cell-cell competition in the intestinal crypts. By exploring the current literature on cell-cell competition in the gut and aneuploidy in early CRC, I aim to shine more light on this understudied topic.

I. CIN and aneuploidy in CRC development

Chromosomal instability (CIN) refers to an increased rate of chromosome mis-segregations during mitosis. CIN can lead to alterations in chromosome number or chromosome structure in the daughter cells. The resultant imbalanced number of whole chromosomes and thus altered karyotype is referred to as aneuploidy. In contrast, structural alterations as a consequence of CIN can be translocations[13], chromothripsis[14,15], duplications, inversions, and deletions, or arm-level aneuploidies[16,17]. CIN can arise through errors in various steps in mitosis, including the premature separation of chromatids, abnormal centrosome numbers, errors in microtubule-kinetochore attachments, malfunction of the spindle assembly checkpoint (SAC), cytokinesis failure, mitotic slippage, or endoreduplication. However, CIN can also arise from errors in managing replication stress, resulting in double-strand breaks (DSBs), errors in repairing these DSBs, and non-allelic homologous recombination[16]. All of these mechanisms have been extensively reviewed before[17].

As mentioned in the introduction, MSS CRC tumors are both CIN and aneuploid, and both features emerge during the early stages of CRC tumorigenesis[18]. However, whether CIN or aneuploidy initiates tumorigenesis remains unclear. This uncertainty arises from the fact that CIN and aneuploidy are closely intertwined. While CIN and aneuploidy often co-occur, modeling pure aneuploidy in vivo, without concurrent CIN, is much more challenging. Furthermore, aneuploidy typically imposes significant cellular stress, leading to fitness disadvantages such as proteotoxic and metabolic stress, as well as replication challenges[19]. Despite this fitness cost, cancer cells must overcome these stresses to survive and proliferate, suggesting a complex relationship between CIN, aneuploidy, and tumor progression.

To explore this relationship, Hoevenaar et al. developed a mouse model to specifically investigate the role of CIN in CRC by inducing varying levels of CIN in APC-mutated mice. This model utilizes Cre-inducible *monopolar spindle 1* (MPS1) knock-in (CiMKi) alleles, which allow precise control over the degrees of CIN. The CiMKi model demonstrated that moderate CIN significantly increased the formation of adenomas throughout the intestinal tract in mice already harboring APC mutations, suggesting that CIN acts as a strong enhancer of tumorigenesis. Furthermore, the findings indicate that CIN can initiate large-scale genome instability and contribute to the early stages of CRC[12]. Similarly, in a study by Rao et al., researchers induced CIN by mutating *budding uninhibited by benzimidazole-related 1* (BubR1) in APC-mutated mice. These BubR1/APC-mutated mice developed ten times more tumors in the colon compared to APC-mutated mice[20]. Both models show that CIN can drive CRC development. However, the specific role of aneuploidy remains unclear, as these studies did not focus on characterizing the karyotypes of the resulting tumors.

Interestingly, a study conducted by Trakala et al. has examined the specific aneuploidies that emerge upon CIN induction, although in a different cancer type – T-cell lymphomas, rather than CRC. The researchers developed a novel mouse model capable of inducing extremely high levels of mis-segregation by excising the *cell division cycle protein 20 homolog* (CDC20) to prevent binding to the SAC. They found that, during early T-cell development, most cells with random chromosomal gains or losses were selected against due to the detrimental effects of aneuploidy. However, this clonal selection eventually gave rise to T-cell lymphomas that exhibited stereotypical karyotypes. A particularly striking finding was that chromosome 15, which harbors the *myelocytomatosis* (MYC) oncogene in mice, was frequently gained in these tumors. The presence of MYC on the gained chromosome 15 seemed to be a key factor driving the selection of this aneuploidy. Furthermore, when these mice expressed human MYC from chromosome 6, the resulting tumors also exhibited gains of chromosome 6[21]. These findings provide direct evidence that the initial detrimental effects of random aneuploidy caused by CIN

can be outweighed by clonal selection, driven by the specific genetic content of certain chromosomes that provide a survival advantage to the cancer cells.

In CRC, recurrent aneuploidies such as gains of chromosomes 7, 8q, 13, and 20q, and losses of chromosomes 8p, 17p, and 18 are commonly observed[22–25]. This suggests that similar selective pressures could drive the recurrence of these specific aneuploidies during tumor development. In fact, a large study about the types of aneuploidy in different tumor types revealed that aneuploidy patterns are tissue-specific[26]. This is particularly relevant in combination with another large study showing that tissue-specific gene expression of oncogenes, specifically those that increased proliferation, can predict which aneuploidy would be selected for during tumorigenesis[27]. These selected alterations are known as driver genes, which promote survival proliferation or adaptation to the tumor microenvironment[28]. The role of driver genes in shaping aneuploidy is often nuanced, with the potential involvement of a strong driver gene (e.g., MYC or TP53) or the cumulative effect of many weak driver genes[28–30]. For instance, in CRC, it might only take one strong driver, whereas in any other cancer, it might take the accumulation of several weaker drivers. This selective advantage potentially allows certain mutations to establish themselves, with drastic consequences to the intestinal tissue environment.

In the next section, I will discuss the concept of cell-cell competition in the context of intestinal tissue homeostasis and then discuss the potential influence of oncogenic mutations, CIN, and aneuploidy in this process.

II. Cell-cell competition in CRC

Tissue health is maintained through quality control mechanisms that allow only the fittest cells to thrive while eliminating those that are damaged or less fit. Although intercellular communication ensures this balance, certain cells can exploit these processes under pathological conditions. A notable example is cell-cell competition, where even healthy yet less fit cells are eliminated by their more competitive neighbors through interactions between them. This phenomenon was first observed in *Drosophila Melanogaster*, in which cells with mutations in ribosomal protein genes proliferated at a slower rate and were outcompeted by surrounding wild-type cells. This observation established that even small fitness differences can determine which cells survive and which cells do not[31]. Subsequent studies in mouse models confirmed that cell-cell competition depends on the relative fitness of neighboring cells, not their absolute fitness[32]. Novel findings led to the concept of ''super-competition,' defined by cells that gain a proliferative advantage and can eliminate wild-type healthy cells that would otherwise survive in a normal context. For example, overexpression of the strong driver MYC has been shown to confer a competitive edge to cells, enabling them to eliminate healthy neighboring cells in both *Drosophila* and murine tissues[33,34]. In the context of tissue homeostasis, cell-cell competition can play an important role in maintaining a balance by promoting or eliminating certain cells. Before exploring this concept further, Box 1 provides an overview of distinct types of cell-cell competition and their defining characteristics[35].

Forms of cell-cell competition

Box 1

Definitions of various forms of cell-cell competition

- **Active vs passive cell competition**: Active cell competition involves cells directly suppressing the fitness of neighboring cells via (I) acquisition of fitness-altering changes, which create a competitive imbalance, (II) recognition of fitness differences through direct or indirect cellular communication, and (III) elimination of less fit cells. By contrast, passive cell competition involves cells outcompeting neighboring cells without direct antagonistic interference.
- **Neutral competition vs biased competition vs super-competition**: In neutral competition, cells compete equally. Biased cell competition occurs when one cell possesses inherent qualities that give it a selective edge over neighboring cells. Super-competition intensifies this by a pronounced dominance that overwhelms neighboring cells.

Cell-cell competition in the healthy gut

In the healthy gut, tissue homeostasis depends on a balance of ISC renewal and differentiation, as briefly mentioned in the introduction. This cyclical process is driven by the interaction between ISCs and their access to various niche factors surrounding the intestinal crypt. Importantly, the architectural organization of the crypt-axis establishes spatial gradients of niche factors, which influence ISC behavior. Examples of these niche factors include Wnt

signaling and Neurogenic locus notch homolog protein (Notch) signaling, which are both indispensable for homeostasis[36].

ISCs proliferate and self-renew and the main pathway regulating that process is the Wnt pathway. In the crypts, high concentrations of Wnt ligands, secreted primarily by surrounding mesenchymal cells and Paneth cells[37], establish a gradient that peaks at the crypt base. This gradient directly stimulates ISCs to divide and proliferate[38]. As progeny cells move upwards toward the villus, they receive decreasing Wnt levels, which facilitates their transition from a proliferative state to a differentiation state[39]. The effect of Wnt signaling on ISCs involves the activation of the β-catenin-dependent canonical Wnt pathway. Upon binding of Wnt ligands to Frizzled receptors, β-catenin is stabilized and translocates to the nucleus, where it activates transcription of target genes that promote cell proliferation.

Notch signaling is another key player in the crypt, where it regulates cell proliferation and fate decisions among ISC progeny cells. The Notch pathway is primarily activated through direct cell-cell interactions, where ligand-expressing cells, such as neighboring Paneth cells and ISC progeny[40], bind Notch receptors on adjacent ISCs. This interaction triggers the cleavage of the Notch intracellular domain (NICD), which then translocates to the nucleus to activate transcription factors that influence cell fate. In the crypts, Notch signaling is essential for ISC proliferation. This was demonstrated in a study conducted in murine which showed that inhibition of Notch signaling causes ISCs and TA-cells to differentiate into goblet cells[41]. Although goblet cells are important for mucus production, the intestinal lining requires a balance of differentiated cell types necessary for other functions like nutrient absorption.

External factors may also play a role in shaping the niche. For example, a calorie-restricted diet has been shown to increase the number of functional ISCs, thereby accelerating clonal dominance within crypts[42,43]. Conversely, aging introduces changes in the niche environment, with aged Paneth cells secreting higher levels of Wnt inhibitors like NOTUM, reducing the fitness of wild-type ISCs[44]. Variations in access to Wnt and Notch signaling also play a crucial role, particularly in the colon, where crypts lack Paneth cells, leading to lower Wnt signaling compared to the small intestine[45]. Thus, the balanced signaling of Wnt and Notch is highly important to coordinate ISC dynamics. However, when mutations arise within ISCs, this balance can be disrupted. This shift from neutral competition to biased competition marks the early stages of tumorigenesis and will be explained in more detail.

Oncogenic mutations in the diseased gut

Oncogenic mutations in key cancer driver genes (APC, KRAS, TP53, and MYC) disturb the balanced competitive environment in intestinal tissues, allowing mutated cells to outcompete healthy neighboring cells. These mutations, studied across various model organisms including *Drosophila* and mouse models, elucidate mechanisms that potentially mirror competitive processes in human ISCs. Before examining the role of each gene in competitive dynamics in the diseased gut, I will first introduce the concept of flower proteins as an overarching concept in fitness-sensing mechanisms (Box 2).

Box 2

Fitness-sensing mechanism: Flower isoforms

One key fitness-sensing mechanism is mediated by the transmembrane protein Flower (hFWE) which exists in different isoforms that communicate cellular fitness. A study in human cells has shown that less fit cells express the isoforms hFWE1 and hFWE3, making them susceptible to elimination, while fitter cells express hFWE2 and hFWE4, signaling their dominance. Further research in mice models, using human colon cancer cells (HCT-116), demonstrated that these cancer cells increased expression of hFWE winner isoforms and induced hFWE loser isoforms in neighboring cells. Interestingly, the expression of only one set of isoforms alone did not drive cell proliferation or elimination; instead, the elimination of less fit cells depended on the presence of neighboring cells. Moreover, blocking hFWE isoform expression slowed tumor growth. Unfortunately, the researchers did not assess potential colon cancer cell mutations, which could provide insights into a potential relationship between isoform expression levels with specific mutations in driver genes. Overall, these findings suggest that hFWE isoforms play a significant role in assessing fitness levels among cancer cells[97].

APC

APC mutations, often among the earliest events in CRC, initiate competitive imbalances within the crypt by enhancing cellular fitness. When APC is mutated, Wnt signaling is continuously activated without the need for ligand interaction. This results in a proliferative advantage for APC-mutant cells, as shown in *Drosophila* and mouse intestinal models, where APC-mutant cells consistently outcompete healthy neighboring ISCs[46,47]. This unregulated Wnt activation enables APC-mutant cells to colonize entire crypts, forming monoclonal crypts that initiate CRC. APC-mutant cells also exploit the recognition of fitness differences within the crypt. Research in mice demonstrated that APC-mutant ISCs secrete Wnt inhibitors like NOTUM, which reduced the ability of neighboring ISCs to proliferate and induced differentiation in neighboring ISCs[48]. This signaling imbalance allows APC-mutant crypts to gain a competitive edge. Studies further reveal that APC-mutated crypts may also exploit paracrine signaling in neighboring crypts to induce differentiation, eliminating healthy crypts and enhancing their dominance through field cancerization[49]. Besides inducing differentiation in neighboring cells, they are also actively eliminated. In the *Drosophila* midgut, APC-mutant cells have been observed to induce apoptosis in neighboring cells through JNK-dependent signaling pathways[50]. This selective elimination mechanism enables APC-mutant ISCs to sustain their advantage over healthy cells. Notably, a study in *Drosophila* suggests that when competitive apoptosis mechanisms are blocked, APC-mutant cells lose their competitive edge, behaving similarly to wild-type cells[51]. This suggests that cell elimination is essential for APC-mutant cells to establish their dominance in the crypt.

KRAS

Mutations in the KRAS gene, observed in nearly 40% of MSS CRC cases, can drive hyperactivation of signaling pathways like *mitogen-activated protein kinase* (RAS-MAPK) and *PI3K/protein kinase B* (PI3K/AKT) pathways, leading to enhanced proliferative capacity[52,53]. KRAS-mutant ISCs gain a competitive fitness advantage by promoting cell proliferation and by remodeling the local ISC niche. In a study conducted in the mouse small intestine, it was demonstrated that KRAS-mutant ISCs secrete *bone morphogenetic protein* (BMP) ligands,

which inhibit neighboring wild-type ISCs from differentiating or self-renewing. This disruption weakens the competitive ability of wild-type ISCs, providing mutant ISCs a competitive advantage[49]. KRAS-mutated cells also participate in recognizing fitness differences through mechanical stress or tissue crowding, which occurs when epithelial cells accumulate within a restricted space[54]. A study conducted in *Drosophila* indicates that RAS-mutant cells exert mechanical pressure on wild-type cells, forcing them into apoptosis via increasing TP53 levels[55,56]. This phenomenon of mechanical cell-cell competition has been observed in human and mouse epithelial tissues as well, suggesting it may be a conserved competitive mechanism across multiple species[57].

TP53

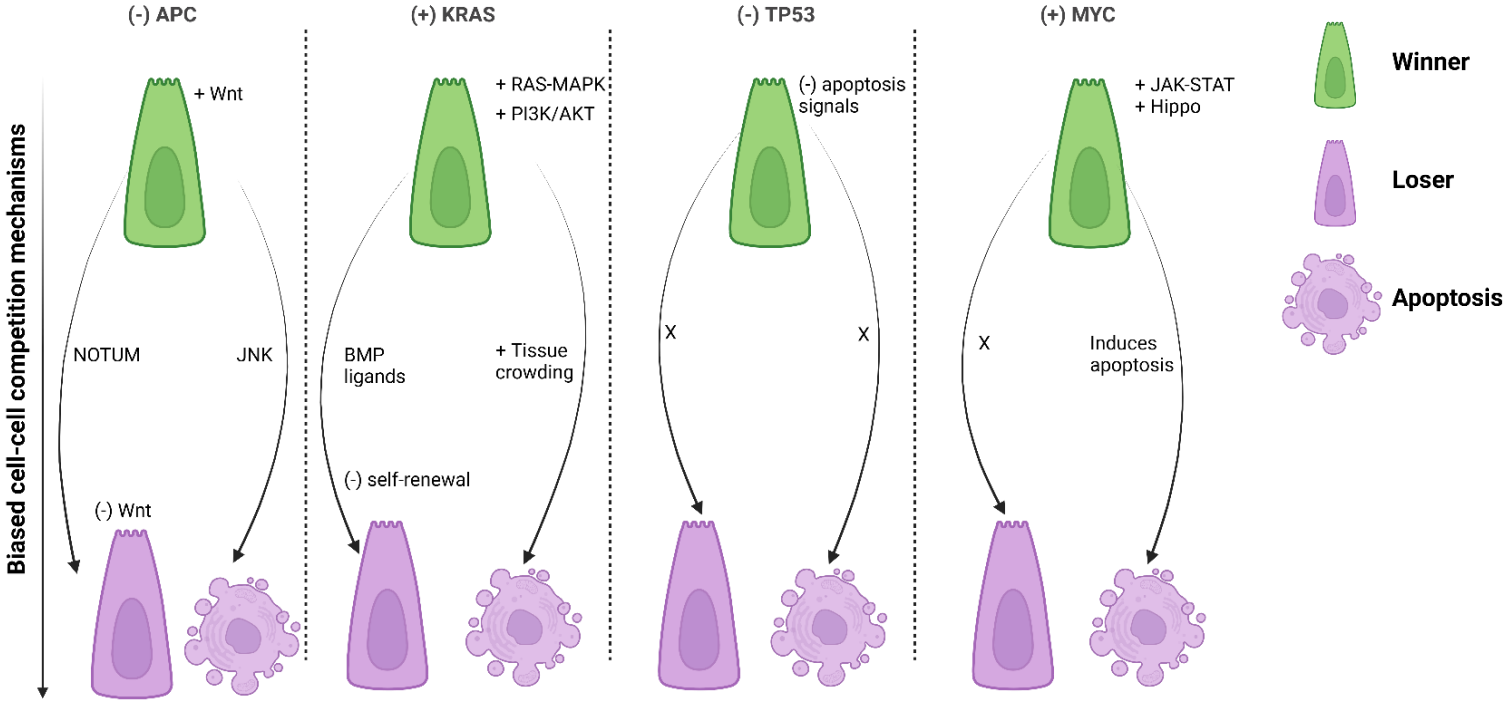
TP53 is a well-known mediator of apoptosis and cellular repair and mutated in 40-50% of CRC cases[58]. Mutant TP53 cells exhibit impaired apoptotic responses, enabling them to survive conditions that would typically lead to cell death in healthy ISCs. This resistance to apoptosis provides TP53-mutant cells with a competitive advantage, especially under conditions of inflammation. This result supports another study that demonstrated that mice on a high-fat diet were unable to extrude oncogenic RAS-mutated cells, due to a reduced fitness of wild-type ISCs[59]. TP53-mutant cells further engage in fitness recognition by utilizing *epithelial defense against cancer* (EDAC), which works to suppress the expansion of oncogenic cells by eliminating them before they can establish themselves in the crypt[60]. In *Drosophila,* EDAC has been shown to target TP53-mutant cells, extruding them from epithelial tissues to prevent cancer progression[61,62]. One important factor that influences the outcome of EDAC is the relative fitness of the surrounding wild-type cells, which are weakened in initiating CRC. Interestingly, a study conducted in *Drosophila* has shown that upregulation of TP53 is required to eliminate wild-type neighboring cells[63], which suggests that a loss of TP53 in early stages of MSS CRC development would impair cell-cell competition.

MYC

The overexpression of MYC shifts cellular metabolism toward aerobic glycolysis, giving MYC-overexpressing cells a super-competitor status. This reprogramming is known as the Warburg effect, which describes how cancer cells reprogram their metabolism[64]. This provides more energy to cancer cells, which they require for rapid cell division. MYC-overexpressing cells recognize fitness differences by activating pathways like JAK-STAT and Hippo. A study in *Drosophila* wing cells has shown that STAT activity is linked to competitor status. Cells lacking STAT activity were outcompeted by neighboring wild-type cells, whereas cells overexpressing STAT become super-competitors eliminating neighboring cells[64]. In *Drosophila*, cells overexpressing MYC eliminate neighboring wild-type cells by upregulating glycolytic pathways, essentially outcompeting them at the metabolic level[33].

In summary, the acquisition of mutations in various genes in ISCs can be important to CRC initiation by providing a competitive edge over wild-type ISCs (summarized in figure 1). Besides mutations in oncogenes, MSS CRC is characterized by recurrent aneuploidies like the gains of chromosomes 7, 8q, 13, and 20q, and losses of 8p, 17p, and 18 (referred to in section 1). Interestingly, aneuploidies such as gains in chromosomes 7, 13, and 20 are already present in early adenomas[65], and their frequencies increase as the tumors progress to carcinomas. This suggests that these aneuploidies may confer a competitive advantage. Below I will explore how CIN and aneuploidy could affect cell-cell competition.

Figure 1. Schematic overview of oncogenic mutations on cell-cell competition



ISCs acquire common oncogenic mutations (APC, KRAS, TP53, MYC) in MSS CRC development. Each column outlines an individual mutation, as displayed on the top. The symbols adjacent to the genes mark either a loss-of-function (-) or a gain-of-function (+). Furthermore, cells in green depict winner cells, whereas cells in purple depict loser cells. The blobbed purple cell indicates apoptosis (see the legend). The arrows from winner to loser cells indicate the biased cell-cell competition mechanisms at play.

The potential impact of CIN and aneuploidy on cell-cell competition

Aneuploidy imposes substantial cellular stress, commonly reducing cellular fitness due to proteotoxic and metabolic stress. These stressors can make it difficult for aneuploid cells to thrive, especially when fitness disparities can be sensed by neighboring cells and acted upon. However, as aneuploid and non-aneuploid cells co-exist and lead to the expansion of aneuploid cells, aneuploid cells are likely selected for due to an enhanced fitness advantage[66]. Studies have shown that only aneuploid cells with simple chromosomal alterations, like partial chromosome gains, manage to persist almost only when TP53 was dysfunctional[67,68]. Without selective pressure, cells with aneuploidies struggle to expand.

In embryonic development, there is evidence that organisms possess mechanisms to detect and selectively eliminate aneuploid cells, ensuring proper development. For instance, studies in mouse embryos have demonstrated that aneuploid cells, induced by SAC inhibitors, are actively selected against and removed[69]. This elimination is largely driven by the TP53 pathway, but only when these cells are in proximity to healthy fitter cells[70]. Physiological consequences of aneuploidy, such as proteotoxic stress caused by imbalances in gene expression and protein levels, may serve as signals that these cells are unfit and must be removed[67]. One model, first described in Drosophila, suggests that aneuploid cells with imbalances in ribosomal protein (Rp) dosage – leading to deficiencies in ribosome assembly and protein translation – are selectively targeted for elimination through cell-cell competition[71]. Cells deficient in Rp gene expression exhibit impaired ribosomal function and slower growth, which results in a competitive disadvantage compared to healthy neighboring cells. The Xrp1 pathway, a key regulator activated in response to Rp gene insufficiency, mediates the elimination of these weaker cells by sensing their reduced cellular fitness. This mechanism raises questions about whether similar pathways operate in aneuploid mammalian cells. In mammals, where there is no direct ortholog of Xrp1, the *DNA damage-inducible transcript 3* (DDIT3) and TP53 are thought to perform similar functions[72,73]. The involvement of TP53 in eliminating aneuploid cells in mouse models supports the idea that a conserved mechanism exists for recognizing and eliminating aneuploid cells in mammalian tissues[70]. In short, while aneuploidy typically imposes a fitness penalty, recurrent chromosomal gains and losses observed in CRC suggest that specific aneuploidies might provide selective advantages. These advantageous alterations may allow cells to thrive under certain conditions in the ISC niche. Within this niche, selective pressures may help aneuploid cells gain a ‘’winner’’ status, particularly when carrying specific oncogenic mutations, which I will further explore below. Importantly, as little information is known about the topic, I engage in hypothetical scenarios, which could provide further insight into the mechanisms at play.

Specific chromosomal gains and losses can provide unique advantages. For instance, the loss of tumor suppressor genes on chromosomes 17p (e.g. TP53) and 18q (e.g. SMAD2, SMAD4) may enable cells to evade apoptosis and foster their progression toward invasion[74]. Similarly, a common early aneuploidy is the gain of chromosome 7. A gain of chromosome 7 has been associated with many types of cancer, including 60% of MSS CRC cases, and has been associated with increased cellular proliferation by enhancing EGFR expression[9,75,76], making it a potential driver that sets the stage for further mutations and aneuploidies. Importantly, using definitions such as ‘drivers’ has to be conducted carefully, as no correlation between the APC gene mutation and the gain of chromosome 7 has been established.

The early gain of chromosome 7, which harbors the EGFR gene, may further contribute to cell-cell competition, especially when oncogenic mutations that lead to rapid proliferation are present. For example, rapid proliferation leads to increased mechanical pressure by compressing neighboring tissue and eliminating healthy cells even several layers away via apoptosis through compaction-driven downregulation of EGFR signaling in healthy cells[77]. Eliminated cells leave space for chromosome 7-mutated cells to expand within and extrude from the crypt. Intriguingly, a study conducted in stem cell-derived murine colonic epithelial organoids showed adaptation to EGFR withdrawal via EGFR inhibition using the drug Gefitinib. These adapted cells demonstrated dysplasia, increased proliferation, and the ability to survive independently of niche factors, all of which were associated with the onset of aneuploidy and chromosomal missegregation[78]. However, the specific gained and lost chromosomes were unidentified and it remains a question whether EGFR-encoding chromosomes were selected for. Hypothetically, the acquired aneuploidy should provide EGFR-overexpressing cells a competitive advantage over their wild-type neighboring cells, allowing them to expand within crypts. Additionally, EGFR overexpression has been shown to support clonal expansion in lung cancer cells[79].

Similarly, other recurrent alterations, such as the loss of chromosome 5q, where the APC gene resides, are observed. Although the APC gene first acquires a mutation, it can later be completely lost. Analysis of the APC gene reveals that its protein performs many critical functions, including DNA repair, cell migration, adhesion, proliferation, differentiation, chromosomal segregation, and mitotic progression. Most APC mutations in CRC tumors lead to truncated proteins, impairing these functions and potentially enabling cells to evade regulatory controls. For example, truncated APC proteins have been shown to impair base excision repair and alter double-strand break (DSB) repair and DNA damage response (DDR) pathways, allowing cells to accumulate additional genetic mutations[80,81]. Thus, a single mutation might initially lead to enhanced proliferation and may be sufficient to become niche-independent. Hypothetically, selective pressures can then lead to the full inactivation of APC through the loss of chromosome 5q and further disrupting all its other functions with drastic consequences. Also, the gain of chromosome 8q, which harbors the driver MYC gene, further promotes tumor progression through metabolic reprogramming and accelerating proliferation[82]. A study conducted in renal cell carcinomas demonstrated that the gain of chromosome 8q correlates with MYC upregulation, which might result in increased proliferation and potentially give them a competitive advantage over wild-type cells[83]. However, excessive MYC expression can also trigger apoptosis through mechanisms like JNK signaling[84]. Interestingly, as opposed to wild-type cells surrounded by EGFR-overexpressing cells, wild-type cells surrounded by MYC overexpression are also induced to undergo apoptosis due to JNK signaling[85]. This should provide more space for cancerous cells to expand and outcompete their neighboring cells. A study in human pluripotent stem cells (hPSCs) reveals that cells with higher MYC expression and lower TP53 activity are better able to proliferate despite aneuploidy, underscoring the importance of these mutations in overcoming the inherent fitness costs of an abnormal karyotype[86].

In conclusion, although significant insights exist regarding the effects of oncogenic mutations on cell-cell competition, the roles of recurrent aneuploidies in CRC initiation and progression remain less clear. Recurrent chromosomal gains and losses suggest that these aneuploidies may confer a competitive advantage under selective pressures present within the ISC niche. However, additional research is necessary to fully elucidate how these aneuploidy patterns contribute to cell-cell competition and survival within the niche. In the next section, I will elucidate how the effects of recurrent aneuploidies on cell-cell competition can be studied.

III. Methods of studying CIN and aneuploidy in cell-cell competition

Human cancer cell lines are essential tools for the study of aneuploidy, *in vitro*. Through CRISPR/Cas9-based engineering, techniques like KaryoCreate have enabled researchers to induce specific aneuploidies[87]. For instance, engineered loss of chromosome 18q in pilot experiments has been shown to promote tumor growth, consistent with prior studies on chromosome 18q[74,88]. In these two-dimensional (2D) systems, co-culturing wild-type and aneuploid cells in mixing assays allows for the examination of competitive dynamics. These models have been used to understand how cell-autonomous effects, such as apoptosis, proliferation, or senescence, play into cellular competition. However, the limitations of 2D cultures – such as their inability to mimic the complexity of tissue interactions – require the use of more advanced models for studying cell-cell competition in an environment that resembles *in vivo* intestinal tissues.

Three-dimensional (3D) organoid cultures derived from ISCs offer a more complex system that better resembles *in vivo* tissue architecture, providing insight into the behavior of aneuploid ISCs within crypt-like environments. This complexity is important, as interactions between aneuploid cells and the surrounding tissue may be key in determining whether aneuploid cells gain clonal dominance and expand within the crypts. Organoids can be engineered to harbor specific chromosomal alterations, allowing direct visualization of competitive interactions between wild-type and aneuploid cells in the crypt. Such models facilitate the study of how altered cells compete with or are eliminated by neighboring cells, providing insight into the mechanisms underlying clonal selection. For example, through CRISPR-Cas9 gene editing, targeted aneuploidies can be introduced in organoids, and lineage tracing can then track the clonal evolution of these altered cells over time[89,90].

Advanced *in vivo* models, such as Genetically engineered mouse models (GEMMs), enable further exploration of cell-cell competition within the fully developed intestine. GEMMs can replicate CIN or recurrent aneuploidies observed in human CRC. To induce CIN, disruptions in the chromosomal segregation machinery have to be made. These disruptions include using drugs like Mps1 inhibitors that interfere with the mitotic checkpoint, to ensure that cells enter anaphase without proper microtubule attachment[91]. Another disruption includes interference with microtubule dynamics by drugs such as Taxol and Nocodazole[92]. Such drugs have allowed the development of inducible CIN models to precisely control the level and duration of chromosomal missegregation[21,93]. By selectively inducing CIN within crypts, researchers can create a mosaic of aneuploid and diploid cells, which can be analyzed to observe how different aneuploidies impact clonal competition.

Advances in molecular event recording[94,95] and single-cell sequencing[96] have revolutionized the understanding of clonal competition. Molecular event recording involves marking specific chromosomal or cellular stress events, such as DNA damage response activation, directly into the genome. Tracking of cell fates enables researchers to potentially reconstruct event histories of aneuploid cells and examine how specific chromosomal alterations confer competitive advantages and might be useful in understanding selective pressure within the ISC niche. When combined with imaging techniques like fluorescence *in situ* hybridization (FISH) and image-based flow cytometry, these models allow precise tracking of chromosome gains or losses and provide detailed insights into the effects of CIN on cell-cell competition within an intestinal environment.

As research progresses, integrating advanced techniques from different biological fields and computational modeling of clonal expansion will further elucidate the role of aneuploidy in cell-cell competition. For instance, mathematical modeling of clonal dynamics has already shed light on the evolutionary pressures that shape competition within tissues[51]. This can be

particularly insightful when simulating selective advantages associated with specific aneuploidies. Integration of computational approaches with single-cell genomic data from engineered organoids or GEMMs offers a powerful research flow for understanding how recurrent aneuploidies contribute to cell-cell competition in the intestinal crypt.

In summary, while significant developments have been made in understanding cell-cell competition in CRC through engineered cell lines, organoid models, and GEMMs, the precise role of recurrent aneuploidies and its potential contribution to clonal dominance within the intestinal niche remains an active area of research. Advanced techniques in molecular recording, lineage tracing, and computational modeling provide powerful tools to reveal the selective pressures acting on aneuploid cells and how these contribute to clonal evolution in CRC. Further research integrating these models will be crucial in ultimately enhancing our understanding of CRC development.

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