POSSIBLE INFLUENCES ON THE DIFFERENCE IN MUTATION RATES BETWEEN CIN AND MIN COLORECTAL CANCERS

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Abstract
Colorectal cancer (CRC) is the fourth most common cancer type of cancer diagnosed in the western world. Genetic instability is a relevant source for both genetic and phenotypic diversity in this cancer. Oncogenes and tumour suppressor genes are often mutated and result in increased mutation rates which in turn, result in carcinogenesis. This genomic instability occurs through several pathways, in which two are discussed in this paper. One type of CRC results from chromosomal instability (CIN), which is due to the miss-segregation of chromosomes during mitosis, known as aneuploidy. CIN occurs in 85% of the colorectal cancers. The other 15% is due to microsatellite instability (MIN), which results from a defect in the mismatch repair system during cell division. These two mechanisms are widely studied and differences in phenotypes, mutations, prognoses and mutation rates have been analysed. However, studies in differences between progression rates are difficult to find, but could still be important for understanding certain CRC mechanisms. However, research has revealed extensive differences within MIN tumours and CIN tumours. For example, MIN mutation rates can increase with increase in microsatellite length, which has less association with CIN tumours. MIN CRC shows better prognosis than CIN CRC, which might be a result of a lower progression rate. In addition, MIN tumours tend to occur more frequently in earlier stages than CIN tumours, which are often seen in stage II and III colorectal cancer. The probability for a MIN-associated MMR defect is 0,01% per cell division, whereas the chance that a chromosome miss-segregates and therefore CIN occurs, is ~25%. This suggests that CIN occurs 250 times more frequently than MIN. Unfortunately, CRC is a very complex disease where differences arise between CIN and MIN tumours, heterogeneity, genes, tissues, stages and origins of the cancer. Therefore, this extreme diversity makes it a challenge to make specific comparisons between CIN and MIN, but not less important.

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Introduction

Genetic instability is an important source for genetic and phenotypical diversity within and between different cancers. It can arise through several pathways and is able to contribute to several stages in the development of tumour growth. Cancer is a worldwide studied disease, but with still a lot of question marks regarding its biology. Mutational frequencies of oncogenes and tumour suppressor genes vary between different tumours within the same cancer. Colorectal cancer (CRC) is one of the best understood solid human cancers. The tumour suppressor gene APC and the oncogene BRAF are two commonly mutated genes in colorectal cancers [1]. A major driving force for the progression of tumours is heterogeneity [2]. Individual patterns of both single nucleotide variations and copy number variations can vary to a large extent between and within tumours. But still, a lot remains unknown in CRC. Questions concerning the number of genes and their mutation rates, progression rates of cancer, differences between primary and metastatic lesions are still yet to be answered. What we do know, is that colorectal cancer can either show chromosomal instability (CIN), which is a consequence of miss-segregation of chromosomes from mother to daughter cells [3]. On the other hand, colorectal cancer can also occur through microsatellite instability (MIN), which are sequences of repeated nucleotides in the genome. Due to a defect in the mismatch repair (MMR) system during DNA replication, MIN can cause tumour progression [4]. CIN and MIN are both associated with genomic instability, but with different pathways. By cancelling out specific genes responsible for either MIN or CIN, researchers have revealed that these two instabilities are strongly negatively correlated [5]. Even by cancelling out certain MIN genes, certain cells showed CIN phenotypes [6]. Additionally, according to several researchers, MIN cancer tends to show better prognosis than CIN cancers [5,7]. This makes CRC a complex disease, because every single case is typical by its distinctive genetic background. By classifying similar tumours, certain types of pathogenesis and the biological behaviour of a specific tumour might be able to predict in the future.

As cells progress towards cancer, they accumulate large fraction of mutations to gain a selective growth advantage. To contain this advantage, cells are likely to show genetic instability in attempt to increase their fitness [8]. In addition, the contribution of mutations and their frequencies (i.e. mutation rates) differs between genes. A lot of CRC studies have experimented prognosis, gene and mutation involvements, phenotypes, heredity and certain rates between CIN and MIN colorectal cancer. However, experiments on specific differences between progression rates are difficult to find. This might be for a reason, because CRC is extremely a complex disease and priority may lay on different topics. In this paper, the

differences between CIN and MIN cancer will be discussed, with focus on possible influences on progression rates, particularly in colorectal cancers.

Microsatellite Instability pathway

Replication of a cell must take place before it can produce two genetically identical daughter cells. Because DNA in cells is repeatedly damaged by environmental and internal influences, it is very important that the of repair of this genetic information is maintained. Normally, this information can be stored stably because of a large set of DNA repair systems, such as the mismatch repair system (MMR). The MMR system provides a proofreading system that removes replication errors [31]. Unfortunately, when mutations occur in the MMR genes, it can lead to DNA sequence mismatches during replication. These mutations may positively or negatively affect protein function by changes in transcription or gene silencing [10].

Moreover, a microsatellite is a sequence of single repeats (SSRs) in both coding and non-coding regions, varying in the length of two to five nucleotides. Microsatellite instability (MIN) occurs as a spontaneous loss or gain of nucleotides in these DNA sequences, due to the defect in four interacting MMR proteins (MLH1, PMS2, MSH6, MSH2) [9]. Additionally, this leads to the accumulation of frame-shift mutations in the microsatellites and therefore, leads to genomic instability [11]. According to H. Ellegren, two different mutations can operate on the microsatellite repeats: length mutations and point mutations [12]. The higher the increasing repeat length, the higher the length mutation rate. As for point mutations: long repeat arrays are broken into smaller pieces. This accumulation of frame-shifts causes mutational inactivation of different MMR genes, which in turn, are important for tumour suppressive functions. Thus, both mutations can lead to carcinogenesis of tumours in humans [4]. One well-studied MIN phenotype occurs in the Lynch Syndrome, which contributes to the Hereditary Non-Polyposis Colorectal syndrome (HNPCC). This is a heritable form of colorectal carcinoma (CRC) in humans, due to a spontaneous mutation. About 90% of the HNPCC syndromes show forms of microsatellite instability [4]. Not only for colorectal cancer, but also the higher risk for other sorts of cancers (i.e. gastric, lung, endometrial), arise in Lynch Syndrome [11].

The colorectal tumour MIN phenotype is associated with high histological grade, tumour-infiltrating lymphocytes and more necrosis, compared to normal cells [11].

Still, it remains widely unknown which exact mechanism contributes to the favourable prognosis of MIN tumours. Although, Sinicrope et al. stated that CRC patients with MIN tumours had a significant improvement in disease-free survival and overall survival in

comparison with CRC patients with microsatellite stable (MSS) tumours, which is thought to be the opposite of MIN [7]. A possibility is that this is due to the high number of tumour-infiltrating lymphocytes in MIN tumours, discussed earlier. Another study performed by Gerbert et al. (2005), suggests that high microsatellite instable CRCs elicit a protective host response and therefore, might prevent the formation of metastasis [13]. Additionally, by comparing tumours with a deficient MMR system with tumours with a proficient MMR status, Sinicrope et al. found that MMR deficient tumours had a delay in recurrence time [7]. A delay in recurrence time could suggest a decreased progression rate. Several studies have supported the idea that point mutations break up perfect repeats and reduce the mutation rates of microsatellite loci [12].

A study in 1999 (Tomlinson et al.) stated that MIN functioned as a sign of raised mutation rate, and however, is not per se carcinogenic. Therefore, one must focus on target structures in coding regions or regulatory regions of genes, such as MMR genes [30]. Therefore, high replication rates in MIN cancer are not necessarily associated with microsatellite instability itself, but a certain mechanism associated with it.

These findings might suggest that MIN tumours provide advantage over other cancer types that show MSS.

Chromosomal instability pathway

Each time a cell divides, its genome is duplicated. During mitosis or meiosis, the DNA is evenly distributed over the daughter cells and they therefore share the same number of chromosomes as their mother cell. If this process fails to take place, this could lead to the unequal distribution of chromosomes to daughter cells, known as aneuploidy. Aneuploidy causes a growth disadvantage in normal cells which in turn can cause, for example, miscarriages, mental retardation and Down's syndrome [14]. Likewise, tumour cells tend to show aneuploidy, which is a cause of chromosome miss-segregation leading to an abnormal karyotype. This is in contrast with normal cells, suggesting that tumour cells show high vitality instead of a growth disadvantage. This miss-segregation is called chromosomal instability (CIN), which is an increase in the rate of gains and losses of whole chromosomes during mitosis [3]. A theory for the origin of CIN is that the chromosomal instability phenotype seems to be dominant, as it can be conferred to a chromosomally stable, diploid cell [8]. Importantly, aneuploidy and chromosomal instability do not share the same definition: CIN refers to a certain rate, aneuploidy refers to an abnormal chromosomal count.

Wide-spread research has shown that aneuploidy is a hallmark of most solid tumours [15]. Three most common mechanisms of CIN tumour cells are: up- and downregulation of mitotic spindle assembly checkpoints (SAC), centrosome over-duplication or defects in sister chromatid cohesion [15]. Before the anaphase takes place during cell division, the SAC ensures that all kinetochores are attached to the spindle microtubules properly [16]. Deletion of SAC genes results in aneuploidy and therefore, a decreased fitness of the cells. In striking contrast, many human cancers exhibit extensive levels of aneuploidy, which suggests a toleration for aneuploidy by cancer cells [16]. Torres et al. demonstrated the existence of aneuploidy-tolerating (UBP6) mutations which contributed to the increase in cell fitness in yeast strains[14]. Another important feature of CIN is, that it leads into increased genetic intra-tumour heterogeneity, a state in which tumour cells can show distinct morphological and phenotypic profiles [17].

Essentially, chromosomal instability drives the progression of tumours through accelerating the loss of tumour suppressive genes and therefore increases carcinogenesis. In addition, CIN is also considered to promote tumour formation through copy number gains of oncogenes [18]. As for the prognosis, Carter et al. identified the occurrence of chromosomal instability in six different cancers and found that this occurrence was higher in metastasis samples than in primary tumours [19]. This might suggest a poor prognosis for patients with CIN-associated cancer. Moreover, studies using mouse models have revealed that rates of CIN tumour formation varies between organs, in which the most in lungs, liver and lymphoid organs. Additionally, CIN-induced tumorigenesis is highest for carcinomas, compared with lymphomas and sarcomas [15]. This suggests that, in addition to MIN cancer, both CIN and MIN cancer contribute to high proliferation rates.

MIN & CIN in colorectal cancer

One of the most common causes of cancer deaths in the western world is Colorectal Cancer (CRC). Interestingly, this form of cancer arises throughout several pathways, as discussed. Genomic instability, which is expressed in both chromosome instability (CIN) and microsatellite instability (MIN), is a cell-autonomous trait, in which one mutant cell causes other cells to exhibit a mutant phenotype. This provides a developing tumour cell with genomic and mutational plasticity which makes it survive, proliferate and disseminate[15]. Almost all mutations in colorectal cancer are associated with MIN and CIN pathways.

These pathways are strongly negatively correlated with each other and both associated with different mutations [5]. This might be since, when a cell shows one instability state, there is no need for another.

As discussed earlier, the microsatellite pathway (MIN), due to a defect in mismatch repair machinery, results in a higher probability for insertions or deletions in the microsatellites and accounts for 10-15% of the sporadic CRCs [5]. Sporadic MIN tumours occur most often in the right colon and tend to show poor differentiation and increased infiltrated lymphocytes. Interestingly, MIN cancers have been reported of showing a good prognosis in stage II and III in colorectal cancers [5].

The other 85% of CRC arises through the chromosomal instability pathway, which is due to the uneven distribution of chromosomes to daughter cells during mitosis [20]. The exact mechanism of causes of this uneven distribution remains unidentified, but tends to have a correlation with a defect in the spindle assembly checkpoint (SCA) during mitosis [5]. One of the causes of colorectal cancer is a mutation in the APC tumour suppressor gene, and contributes to cancer pathways in de colon [8].

Microsatellite stability (MSS) seems to occur in 80% of the bowel cancers [21]. As discussed above, 85% seems to be associated with CIN. Another study by Razvan et al., showed that CIN phenotypes were enriched in MSS subtypes. However, differences in terms of genes, prognosis and molecular mechanisms were also found [26]. Nevertheless, CIN and MSS seem to show certain similarities. In contrast, Hause et al. observed correlations between the frequency of MIN events in high-MIN and MSS malignancies of the same cancer type and across types. This could suggest related instability patterns within and across malignancies [10].

At the level of glands, which are the smallest units of colorectal premalignant lesions, MIN might exist at a higher rate than previously observed [25]. Higher progression rates to cancer have been detected in MIN tumours compared to MSS tumours, but not consistently. Beggs et al. stated two possibilities for this occurrence [25]. First is the possibility of a clone of microsatellite unstable glands within a MSS tumour, which may confer a selective growth advantage for tumour development, even though the tumour does not seem to show MIN characteristics. A second explanation could be that MIN is a “background” phenomenon and does not influence the progression to cancer, but is only present in that tissue. It is also possible that cancer cells develop chromosomal changes at the same rate as normal cells, but that gross chromosomal changes are lethal to normal cells, but not to cancer cells.[8]

Cancer onset

The whole process, from the occurrence of a first mutation in CRC cancer, to the initiation of tumour genesis and metastasis respectively, can take twenty to fourty years [8]. An explanation that tumours are often heterogeneous, is due a continuing accumulation of genetic changes taking place throughout these years. At some point, genetic instability takes place. Rajagopalan et al. discussed that the timing for CIN and MIN to occur in early tumours are possibly consistent towards each other [8].

In addition to MIN CRC cancer, the outcomes of various studies have shown a strong positive association between MIN and methylated promotor CpG islands (CIMPs). A number of tumour suppressor genes, such as TGF-β, have been shown to be silenced due to this methylation, which is an important mechanism in human carcinogenesis [18].

Almost all cancers that occur sporadically, show levels of MIN, but in most of them MIN is not considered a great influence. In contrast, MIN is found in only 15% of the colorectal cancers as a result of somatic mutations in MIN-associated MMR genes [21]. These mutations were found in high grade dysplasia and increasingly in adenomas with first signs of invasiveness [22]. Therefore, mutations due to MIN are found earlier in the development of colorectal cancer. In the germline mutated MIN pathways, genetic instability is shown to occur at the benign adenoma stage in more than half the tumours in HNPCC, but Brueckl et al. proved this wrong in the sporadic form of CRC. Only a small group of tumours showed MIN at early stage in these adenomas [21]. Therefore, questions raise concerning the onset of MIN between stages of tumour progression in colorectal cancer.

Gene & protein expression

Using colorectal cancer cell lines, Dunican et al. found six genes that were consistently differentially expressed in MIN and CIN cell lines [20]. BTF3 (transcriptional activation), H2AZ (nucleosome assembly) and PTPD1 (gene/protein expression) were overexpressed in MIN cell lines. They suggested a chromatin remodelling state which in turn, could affect gene transcription resulting in gene silencing. PTPD1 shows a function in the progression of tumours and loss of heterozygosity (LOH) in specific alleles, which is regularly seen in CIN phenotypes [20]. This implicates possible associations between CIN and MIN CRCs. A suggestion for the cause of the MIN phenotype might be due to certain increased degradation of mutant transcript caused by mRNA instability.

The remaining three genes PLK, RanBP2 and CCNA2, were overexpressed in CIN pathways. Whereas RanBP2 induces chromosome disjunction in mitosis, PLK influences chromosome

segregation and therefore both contribute to chromosome instability. CCNA2 tends to regulate cell cycle checkpoints and therefore damage DNA checkpoints [20]. These findings therefore, implicate differences in genes between CIN and MIN, but also certain associations. Another interesting finding in CRC was the identification of a protein, lamin B2, performed by Kuga et al [6]. They compared protein expression profiles in both MIN and CIN colorectal cancer. By repressing lamin B2 levels in MIN cancer cells, these cells resulted in showing aneuploidy, chromosome miss-segregation and defects in spindle assembly, which are typical CIN cancer phenotypes. In addition, expressing lamin B2 levels in CIN colorectal cancer cells, these phenotypes were prevented [6]. Unfortunately, differences in mutation or progression rates were not analysed. As for the research by Dunican et al., whom found six genes that were differently expressed in CIN and MIN colorectal cell lines, only known genes were examined. In addition, mutation of the same gene in both CIN and MIN cancer tended to result in better prognosis in MIN cancer than in CIN cancer [5]. This might be due to the different stages in the colorectal cancer because MIN pathways are often more associated with earlier stages than CIN pathways. Another important difference that should be taken in consideration in detecting mutation rates is mutation sort, i.e. de novo, heritable, point mutation vs. defect, somatic vs. germline mutations. This implicates further research that should emphasize mutations in both known and unknown genes that are functional in CIN and MIN colorectal cancer [20].

Origin of mutations

The outcome of an experiment performed by Georgiades et al. in 1999 showed that, instead of one catastrophic event early in tumour formation, chromosome instability shows a continuing state. Plurality and divergence of chromosomal changes occur during tumour growth in time [23]. The arising rate of these chromosomal changes, depends on where the mutation occurred. For instance, if the mutation occurred in a gene responsible for apoptosis, this rate would not be affected. In contrast, if the mutation took place in a spindle assembly checkpoint (SCA) gene, it might affect the rate of chromosomal changes and therefore, tumour formation. Furthermore, CIN pathways contribute to the fine-tuning of its growth characteristics in cancer cells, in respond to changing environments (i.e. metastatic lesions during chemotherapy) [8]. As discussed before, one of the causes for CIN is a mutation in the tumour suppressor gene APC. It remains unknown when chromosomal instability occurs during CRC, but Rajagopalan et al. discussed a possible theory and constructed a matching formula [8]. Because a gene contains two chromosomes, two mutation hits should be considered for

inactivation of this gene. According to Rajagopalan et al., it is likely that the first hit is a point mutation and the second mutation could either be a second point mutation or a loss of heterozygosity (LOH), which belongs to the CIN phenotype [8]. Additionally, it is known that second hit in APC tends to be extremely accelerated in the presence of CIN. This suggests that certain mutations in cancer are ongoing, and others do not need an ongoing state.

Genetic instability

For a cell to enhance long term survival, it needs a high level of genomic stability.
Genetic instability, which can result in or be result of CIN or MIN, consistently results in a progressive phenotype as tumour cells mature. If the genomic instability were to appear in both benign tumours and the following metastatic tumour levels, when detecting, there would still be differences in the number of genetic changes. With every wave of clonal expansion, alterations accumulate and become 'locked' in the tumour cell [8]. Thus, quantification of these mechanisms becomes limited because a great variety of generations can have occurred between the time of detection and the last bottleneck in tumour growth. Additionally, the variation in microenvironments that is encountered by the tumour cell throughout its total development, can differ extremely between cancer cell lines. This makes it difficult to construct a consistent genetic profile of colorectal cancer.
It is thought that genetic instability does not arise at the onset of CRC, but somewhere throughout the development of tumorigenesis [8]. This results in the acceleration of mutation rate of dividing cells and can therefore be a major accelerator of replication rates. However, evidence for the occurrence of CIN in early tumours is difficult due to the small sample sizes. Another possibility for this limitation could be that chromosomal alterations cannot be found in cells with a "young" lesion [8]. Furthermore, even though MMR deficiency occurs early in the development of MIN tumours, as explained earlier, the resulting changes in simple repeat sequences are observed more commonly in late tumours [8],[21]. Therefore, the onset of an MMR defect might be more relevant in carcinogenesis, than MIN itself.

Prognosis

In contrast with MIN cancer, the CIN tumour cells show more differentiation rates and fewer tumour-infiltrating lymphocytes. Although, before the experiment by Mouradov et al. in 2013, it remained unknown which of MIN ore CIN mutations are predictors of good survival, and therefore a subset of genes were identified as associates of prognosis, in vitro [5]. These findings came with certain contradictions, i.e. mutations in the BRAF-oncogene resulted in

poor prognosis in CIN-associated cancer, but with a good prognosis in MIN-associated cancer.

Another study based on pathological and molecular data in colorectal cancer, using hierarchical clustering for gene expression profiling, Furlan et al. found differences in prognostic values between CIN and MIN colorectal cancers [22]. They separated 126 colorectal cancers into three groups, based on molecular and pathological similarities, whereby group A and B showed MIN and CIN pathways, respectively. MIN CRCs were assessed using methylation status and were associated with good prognosis, whereas CIN colorectal cancers were assessed by detection of LOH and resulted in poor prognosis. Additionally, CIN CRCs were associated with stages III and IV and comprised the most aggressive tumours, compared with MIN CRCs, which were associated with stage I and II tumours [22]. These results support the findings of Mouradov et al., discussed earlier, who found better prognosis in MIN CRC. Therefore, the prognosis outcome could be an important factor in replication rates. MIN cancers tend to result in a better prognosis than CIN cancers. However, MIN phenotypes are often detected in earlier stages of colorectal cancers than CIN phenotypes. CIN is more frequently detected in metastasis samples than in primary tumours [19]. This makes it difficult to make a comparison between CIN and MIN, due to the progression differences. Although, suggesting that CIN occurs in more aggressive phenotypes than MIN, might implicate higher replication rates.

Progression rates

Progression rates of tumour cells can be influenced by a lot of factors, such as mutation rate, which is the rate at which changes in DNA sequences occur. Cancer cells that possess MIN phenotypes tend to show a mutation rate of two to three times higher than observed in normal human cells [27]. Cancer cells that do not express MIN phenotypes, in generally show aneuploidy which is associated with CIN cancer cells, as discussed earlier. In humans, the rate at which a base-change in a nucleotide occurs is $3 / 3x10^9$ nucleotides, per DNA replication [31]. In a genome consisting of $3x10^9$ nucleotides, 1% contains coding DNA[31] and therefore, $3x10^7$ nucleotides occur in coding regions. Furthermore, the MMR system reduces the number of errors by a factor of 100. With a defect in the MMR system, an error occurs in $3 / 3x10^7$ nucleotides. Therefore, every time $3x10^7$ nucleotides replicate, one mutation takes place. The probability that this mutation results in a functional change, is even lower.

Focussing on CIN CRC mutation rates, the probability of losing or gaining a chromosome is ~0.01 per chromosome per cell division, shown in a study by Lengauer et al [28]. Multiplying

this for all chromosomes in the human genome, the probability for a loss or gain would be ~0.23, which means a chance of ¼ per cell division. The human genome, which consists of 23 chromosome pairs, contains 25.000 genes. By this means, one chromosome consists of ~1000 genes. If miss-segregation occurs per ¼ cell division, 250 genes per chromosome have a chance to result in CIN. In summary, the probability that MIN occurs in per cell division is ~0,01% whereas the chance that a chromosome miss-segregates is 250 times higher (see Tabel 1). With despite of the difficulty in comparing CIN and MIN, MIN tends to show lower probabilities for a defect.

	Error % per cell division
1 Chromosome	~25%
1 Microsatellite	~0,01%

Tabel 1 ***Differences in error rates in microsatellites and chromosomes.***

Moreover, Supek et al. constructed a model to identify the DNA mismatch repair variation throughout the genome of 652 tumours, including colorectal tumours[24]. They state that the earlier the mismatch repair system fails in the history of a tumour the better the prediction of loss of regional rate variability in MIN tumours. This suggests that the timing of the defect in MMR system is of importance for the tumour progression.

The differences in progression rates between well-characterized CRCs are still difficult to explain. It can therefore, not be predicted whether an early lesion is premalignant and will develop into cancer. However, genomic instability leading to heterogeneity for MIN and the associated promotor methylation may lead towards an explanation [25]. Progression rates can in addition, be caused or accelerated by high mutation rates. In turn, high mutation rates in colorectal (and uterine) cancers can be caused by the inactivation of a proofreading domain of DNA polymerase [24]. This proofreading is a result of an exonuclease activity that enhances the quality of polymerase, by cutting out incorrectly placed nucleotides. Therefore, inactivation of this domain could accelerate the mutation and progression rates respectively, in cancer development. Other influences on replication rate might be the time the tumour needs to recur, discussed in the MIN cancer sector. MMR deficient pathways seem to delay this time [7], but this has not yet been verified, so more research is needed to experiment certain pathways due to this delay. Essential is the time when genetic instability occurs, which is not always consistent between MIN cancers.

Lastly, a relevant factor that affects mutation rate is microsatellite length. Mutation rate increases with an increasing number of repeat units [12]. This seems acceptable: the more repeat units present, the more opportunities for replication defects. A length-dependent mutation rate explains part of the mutation-rate variation at several scales [12].

Conclusion

Extensive research has been performed in attempt to complete the major puzzle of colorectal cancer. Until now, a lot seems clear, but still a lot remains unknown. In this paper, the emphasis is put on the differences in mechanisms between CIN and MIN cancers, with colorectal cancer in specific. After discussing certain causes, gene expressions, prognoses, proteins and mutation rates, a few suggestions concerning replication rate can be made. Comparing mutation rates between CIN and MIN, the probability that CIN occurs tends to be 250 times higher than the probability of a defect in the MMR system. This finding can therefore contribute in explaining the differences in prognosis and phenotypes.

In general, it is of course important to keep in mind that the cancer type should be taken in consideration. Differences should be made between sporadic and heritable tumours, between primary tumours and metastases. Even between types of adenomas, there are differences in progression rates [21]. In addition, there are several stages within colorectal cancer. Within these stages, progression rates can obviously, differ from each other and specific tissue where the tumours occur is of high importance. Essentially, as the survival rate of patients with high MIN-related cancers is better[29], mutation rate may be a better prognostic factor.

However, it remains challenging to compare chromosomes to microsatellites due to different mechanisms. This all, implicates the importance of further research in progression rates specifically for understanding the underlying pathways in colorectal cancer and in turn, for a possible treatment.

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