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ON

THE ORIGIN OF CANCER

Analysis of two models on cancer evolution

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8/31/2016

Although much research is done focussing on early diagnosis and treatment of cancer, less is known about the origin and evolution of cancer. At the beginning of the 20th century different models were postulated to understand tumorigenesis, like the somatic mutation theory. Aneuploidy as a driver for tumorigenesis was also already mentioned by Theodor Boveri. In current research on the origin and evolution of tumours, the researchers are divided in two teams. The mutator mutation phenotype model suggest tumorigenesis starts when specific mutations occur in mutator genes, normally involved in DNA repair and genomic stability. The tumorigenesis by aneuploidy model explains tumorigenesis by the fact that more than 75% of all solid tumours are aneuploid, thus have an abnormal number of chromosomes and this abnormality is the reason why tumours develop to a detectable tumour. In this thesis both models will be compared based on experimental evidence given by different researchers. The clinical relevance of the models will be briefly explained. This information will be used to answer the question if tumorigenesis and tumour evolution is best explained by the model of tumorigenesis by aneuploidy or the model of mutator mutation phenotype. After comparison of the given evidence, an attempt will be made to combine both models to a model that is corresponding with all the evidence.

Table of Contents

Introduction

Every year, more than 8 million people die from cancer related diseases worldwide [1], and even more people get cancer every year [2]. Although much research is already done, a cure is not yet found. Most researchers are focussing on early diagnosis and treatment of cancer, but less is known about the origin and evolution of cancer. There are different characteristics that define tumours. In the review of Hanahan and Weinberg in 2011 these characteristics are explained and called the hallmarks of cancer [3]. These six characteristics are the ability to resist cell death, sustaining proliferative signalling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality and induction angiogenesis. These six characteristics are necessary to be acquired for a cell to transform and become a cancer cell.

In order to cure or treat cancer, it is necessary to understand the cause and evolution of cancer cells and tumours. Research suggests different models, but definitive evidence is not yet given. Different theories about the cause of cancer were given as early as the 1900s; the somatic mutation theory, as first mentioned by Ernest E Tyzzer in 1916, explains cancer as a consequence of a mutation in a somatic cell [4]. One cell with the correct mutation can cause an entire tumour to develop. This theory contains the premise that a single exposure to a carcinogen is sufficient to start a tumour. After the initial exposure with a carcinogen, only a promoting factor is necessary to evolve the tumour. This can be experimentally investigated when the number of tumours is the same as the number of cells initially mutated. Researchers Berenblum and Shubik found evidence in 1949 supportive of the theory but also against the theory [5]. They found the theory was true for 9:10-dimethyl 1-1:2-benzanthracene, a known carcinogen, but not for mustard gas. Although mustard gas was found to be a mutagen (an agent that can mutate DNA) in 1946 [6] and therefore is supposed to initiate tumours, this was not the result after exposure to the mustard gas, suggesting that one mutation is not sufficient to initiate tumorigenesis.

Building on the model of somatic mutation theory, it was postulated in 1954 that six or seven mutations were sufficient to develop cancerous tumours, which was named the the multi-stage theory [7] . Although evidence is found that in some cases six or seven mutations are indeed enough to cause tumorigenesis [8–10], it is not an explanation for the heterogeneity found in tumours. Heterogeneity is found on multiple levels; tumours consist of cells that have a lot of different mutations [11] and many cells carry different karyotypes [12]. The heterogeneity is found between tumours (inter-tumour) and in a single tumour (intra-tumour)[13,14]. This is difficult to combine with the somatic mutation theory or the multi-stage theory.

In the last decades two new theories have tried to answer the question of tumorigenesis and give an explanation for the heterogeneity found in tumours. The two models discussed in this thesis are the mutator phenotype theory and the tumorigenesis by aneuploidy theory.

The mutator phenotype model suggests tumorigenesis starts with mutations in specific genes. These genes are called mutator genes. When a mutation arises in these genes, a cell can no longer correctly repair damaged DNA and more mutations will occur. After a latent period, enough mutations will be gathered and a detectable tumour arises. This explanation describes why tumours are found to be heterogeneous and there is a latent period before a detectable tumour arises [15,16].

On the other side is the tumorigenesis by aneuploidy theory. When a cell divides, the genetic content must be divided into both daughter cells. A failure in this dividing of the genetic content causes chromosomal instability, which can cause aneuploidy [17]. An aneuploid cell has an abnormal amount of

chromosomes and could therefore possibly have an abnormal amount of proteins and other materials essential for survival and proliferation. In normal cells aneuploidy is detrimental, however more than 70% of the tumours are found to be aneuploid [18,19]. Although aneuploidy was suggested by Boveri in 1902 as a possible cause of cancer [20], this model was put aside when the somatic mutation theory surfaced. Recently, some researchers claim that the mutator mutation phenotype is not sufficient to explain tumorigenesis and tumour evolution and are therefore exploring the possibility of aneuploidy as the cause of cancer again. They state that only tumorigenesis by aneuploidy is a sufficient explanation of the latent period before a detectable tumour arises and the amount of heterogeneity found in tumours [21].

In this thesis, both models will be explained in more detail, the evidence given by researchers will be reviewed and an objective report of both models will be given. This information will be used to answer the question if tumorigenesis and tumour evolution is best explained by the model of tumorigenesis by aneuploidy or the model of mutator mutation phenotype. The models will be linked to their clinical relevance and finally a combination of both models will be proposed which suits all the evidence and current knowledge about tumours, tumorigenesis and evolution of tumours.

Tumorigenesis and tumour evolution

In order to investigate which model fits best in the current knowledge about tumours, the characteristics of a detectable tumour have to determined and what there is already known about tumorigenesis and tumour evolution must be explained. As mentioned in the introduction, there are six hallmarks of cancer [3]. These acquired characteristics are necessary for a tumour to develop. However, there are also enabling characteristics, enabling the tumour to arise. Such an enabling characteristic is the development of genomic instability in cancer cells. The genomic instability generates random mutation, and could therefore induce the necessary changes in the cell to transform. This genomic instability is one of the reasons that tumours have latent periods before they arise and are heterogeneous.

After first contact with agents that induce tumorigenesis, carcinogens, it can take up to a few decades before a clinically detectable tumour arises. This was already shown for example with cigarette smoke and lung cancer in 1979 [22]. Around the beginning of the 20th century, the amount of men smoking increased significantly; however, the incidence and deaths from lung cancer did not increase until the 1920s. This latent period suggests that a lot of mutations or other changes and adaptations must occur before a transformed cell becomes a detectable tumour.

Tumours found in humans are heterogeneous. This heterogeneity is found between tumours (inter-tumour) and in a single tumour (intra-tumour) in mutations in the genes, epigenetically and in karyotypes. [11–14]. This means that there are mechanisms at work to either drive for heterogeneity or there are mechanisms that shut down which normally sustain genomic stability. It is important to know the origin of the genomic instability, because this impaired mechanism causes tumours to be heterogeneous and difficult to treat. Heterogeneity means that no tumour is alike and could be seen as different diseases. These different tumours have for example different amount of proteins, different DNA sequences and different mechanisms of evading apoptosis. When there is one mechanism present in all the tumours, this is a possible target for treatment. However, because no tumours are identical, two tumours cannot be treated the same. This also causes a big range between incidence, prevalence, prognosis and treatment between different tumours [23,24].

Another characteristic of tumours is the ability to metastasize, outgrowing the original organ and spread to other parts of the body. This happens not directly with tumorigenesis but after time, suggesting this is a characteristic that is gained in time. This indicates that cancer cells keep evolving and mutating, something that also can be seen in the heterogeneity of tumours [25]. Despite all of this, the exact driver for the evolution from a transformed cell to a detectable tumour is not yet discovered.

Mutator phenotype

A single base mutation can occur when a cell is replicating its DNA for proliferation. Normally this mutation gets repaired by the DNA repair mechanism. These mutations seem to be random mutations, although research suggests some mutations occur more often if there is a change in the environment and the mutation will be beneficial for the cell survival [26]. Mutagens are agents that have an impact on the DNA in a cell and mutate DNA, such as UV-radiation, X-rays or biological agents as transposons or viruses. Many mutagens are associated with cancer and are therefore carcinogens [22].

When it was discovered that a lot of mutations (>1000) were present in tumour cells, this was a contradiction with the somatic mutation and multi-stage theory [27]. Because a cell normally has a great DNA repair mechanism and a system for genetic stability, mutations do not happen often and therefore cannot account for more than 1000 mutations in a cancer cell [15,28]. The mutator phenotype theory is building on the theory of the somatic mutation and the multi-stage theory, which states that a few mutations can cause tumours [16]. This theory states that when a mutation occurs in a mutator gene it increases the mutation rate of a cell and could become a transformed cell. The mutator phenotype model suggests the following as seen in figure 1:

- After DNA damage through carcinogens, there are mutations in the cells. When a mutation occurs in the DNA repair mechanism or in any other part of the genetic stability maintenance, it is called a mutator mutation.
- After the initial mutator mutation, lots of mutations appear and selection begins.
- Selection is dependent on nutrition, angiogenesis and hypoxia.
- If all the selection criteria are complied, a clinically detectable tumour arises.
- The tumour exists of different clones with different mutations, the tumour is therefore heterogeneous.

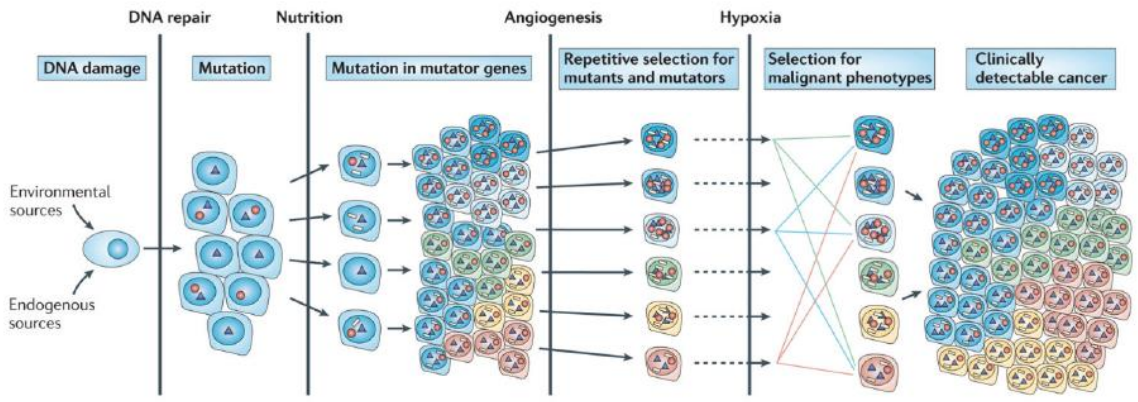

Figure 1. Cascade of mutations during tumour progression in a model of mutator phenotype [16]
Red circles: Mutations in genes that result in enhanced mutagenesis
Blue triangles: Driver mutations that are selected for on basis of microenvironment
White rectangles: Passenger mutations

This model, as is schematically shown figure 1, has a few premises that can be experimentally investigated:

1. Mutator genes have important functions in untransformed cells;
2. Although there is a great deal of heterogeneity, because of all the passenger mutations, a small number of mutations must be seen in all the cells of the tumour. Because the mutator mutation is at the origin of the tumour, it must be present in all tumour cells;
3. Because the origin of cancer cells lies in mutations, carcinogens only functions as mutagens;
4. Mutations are cancer specific;
5. Certain specific mutations are enough to transform normal human cells in cancer cells;

Two more premises are mentioned in the work of Li et al. in 2000:

6. *Cancer phenotypes are as stable as conventional mutations;*
7. *Cancers are diploid, because gene mutations do not depend on karyotype alterations for expression* [21].

Experimental evidence

Every of the premises mentioned above will be reviewed and the experimental data will be provided. When there is evidence against this premise it will also be mentioned here.

Mutator genes have important functions in untransformed cells;

If mutator genes had no important function, these genes would be gone through evolution, because the negative effect outweighs the positive. However, apparently they are still in the genome, thus they should have an important function in the untransformed cell. Although the amount of human mutator genes is not yet discovered, it is found in yeast that there are > 100 genes controlling the DNA repair pathway [29], indicating there are also a lot of genes in humans where spontaneous mutations can occur and increase the mutation rate of the dividing cell, thus acting like a mutator mutation. Some of these genes found in yeast have a human homologue, suggesting a conservation of these genes through evolution. This conservation suggests that these genes have important roles in cell survival. For example the yeast genes MEC1/TEL1, found to be a mutator gene, have human homologues ATR/ATM. ATR and ATM are genes necessary for the repair of DNA after damage [30]. Mutation in these human genes are found in the cancer type ataxia telangiectasia. Another mutator gene found in yeast is RAD53/DUN1 with the human homologue hCHK2. This gene is also necessary for DNA repair, and highly integrated with ATR and ATM. Because of their repair capacity, these genes function in a genetic instability suppression pathway. BRCA1 and BRCA2 (BReast CAncer genes) homologues are not found in yeast, but these genes have direct and indirect interactions with many of the genes found in yeast, suggesting it is also an essential gene in the genetic instability suppression pathway [29].

Although there is a great deal of heterogeneity, because of all the passenger mutations, a small number of mutations must be seen in all the cells of the tumour. Because the mutator mutation is at the origin of the tumour, it must be present in all tumour cells.

The DNA repair mechanism in cells is an accurate system and in normal cells there is no more than one incorrect base for every 10^9 to 10^{10} nucleotides replicated [31]. This is the result of a combination of an accurate base incorporation and exonuclease proofreading by the replicative DNA polymerases Pol δ and Pol ε, and post-replication surveillance by the DNA mismatch repair apparatus [32]. In 2013 the DNA of 15 patients with multiple colorectal adenomas was sequenced. The patients shared mutations in

DNA polymerase Pol δ (POLD1) and Pol ε (POLE)[33]. POLD1 and POLE are units of the proofreading system and therefore Pol δ and Pol ε could be mutator genes.

Another important aspect of correct replication is the repair mechanism after DNA damage. BRCA1 and BRCA2 are both genes that contribute to DNA repair after damage [34]. BRCA proteins are also found to be required for the maintenance of chromosome stability. Moreover, research also suggests there are even more pathways the BRCA genes are connected to, such as the cell cycle [35] and apoptosis after DNA damage [36]. These genes are known to contribute to the onset of breast cancer [37,38] and are therefore possible mutator genes.

However, in one tumour, there is no single mutation that is found in all the cells. This is possible because of evolution of the tumour. When there are enough mutations in mutator genes, it is possible more mutations in these genes are selected against or that even the existing mutations are selected against [27]. A lot of mutations are not beneficial for cells, because it increases stress. Although cancer cells seem to evade the negative effects of this stress, too many mutations will lead to apoptosis. When a mutator gene stays mutated, all the offspring will inherit more mutations. After a while, this will be such a burden for the cell that it is not able to survive anymore. It is therefore necessary that after a while the mutator mutations disappear from the cell. However, a subpopulation of quiescent cells with the mutator mutation can stay behind, although in small amounts. This is clinically relevant, because these subpopulations can create a whole new tumour when the rest of the cells are treated. Another possibility is that because of these mutator mutations, these subpopulations are more resistant to treatment and are therefore a real threat when it comes to curing cancer. It is possible these subpopulations are the so called cancer stem-cells, recently discovered to be a subpopulation in tumours [39]. Nevertheless, there is no evidence these stem-cells have a mutator mutation.

Because the origin of cancer cells lies in mutations, carcinogens functions only as mutagens.
If the origin of cancer cells lie in mutations, every agent that induces tumorigenesis (carcinogens) must induce mutations and are therefore mutagenic. However, there are carcinogens found that are not genotoxic and therefore not mutagenic; asbestos [40], hormones [41], arsenic [42], Ni^{2+} [43] etc. [5,21,44]. Asbestos for example has no genotoxic function, but acts as a carcinogen because it is phagocytosed into the cell and interferes with the dividing of the cell leading to aneuploid cells [40]. Nickel (Ni^{2+}) is found to induce chromosome aberrations and cause chromosomal instability [43]. Arsenic, among other things, interferes with methylation and demethylation of DNA [42]. These agents cause chromosomal instability or interferes with the transcription of proteins and via that pathway could lead to tumours. These findings suggest that mutations are not the only possible cause for tumorigenesis.

Mutations are cancer specific.
Although specific genes are found for specific cancers, like the BRCA1 and BRCA2 gene for breast cancer [38], there are until now no specific mutations found for tumours [45]. Although specific mutations in these genes increase the risk of breast cancer significant, it is not a certainty, suggesting that more than this mutation is needed to initiate tumorigenesis. Also, this specific mutation is not found in all the breast cancers [37]. Despite that, a lot of genes are associated with the risk of getting cancer and the prognosis of cancer, for example the *P53* gene. This gene is commonly known as "*the guardian of the genome*" [46], and is a strong tumour suppressor gene. In 50% of tumours this gene is mutated in some sort [47], suggesting a powerful driver for tumorigenesis and evolution.

Certain specific mutations are enough to transform normal human cells in cancer cells.
In 1999 researches transformed human epithelial cells in cancer cells with the use of three genetic elements. They expressed the telomerase catalytic subunit (hTERT) in combination with two oncogenes [9]. In this experiment these three changes in the genetic elements were sufficient to create tumours. This suggests that certain mutations are indeed enough to transform normal human cells in cancer cells, confirming the mutator mutation phenotype model. However, Peter Duesberg and his colleagues argued that this experiment was not decisive, because the amount of passages needed before transformation (the development of normal cells to cancerous cells) allowed for other genetic alterations to occur, for example aneuploidy [21]. After analysing the cells used, aneuploidy was indeed found in a significant percentage number of tumours. Nonetheless, this evidence gives no answer to whether mutator mutation or aneuploidy is cause or consequence of tumorigenesis.

Moreover, this premise seems to fails to explain the latent period between treatment with a carcinogenic and the rise of a detectable tumour. A mutation in a mutator gene allows a lot of mutations to occur. With this mutation, the amount of mutations in tumours can be easily obtained within less than 20 years, suggesting another change has to be made before cells are transformed. Despite that it is possible that the amount of mutations has no influence on the fact that there are criteria that must be fulfilled before a detectable tumour can arise. As shown in figure 1 the microenvironment has a great influence on the selection criteria. Before a detectable tumour can arise all these driver mutations and the hallmarks of cancer must be acquired and this may take a long time.

Cancer phenotypes are as stable as conventional mutations.
Although most cancers are more or less genetically unstable [48,49], recently it is found that cancers have a stable phenotype and drive towards an equilibrium [50,51]. Although tumours are genetically unstable, they drive towards an equilibrium, suggesting that the mutations are selected for. This is possible when unfavourable randomly acquired mutations drive a cell into apoptosis.

Cancers are diploid, because gene mutations do not depend on karyotype alterations for expression.
It seems logical that expression of mutated genes do not depend on karyotype alterations. However, this is only the case when the mutation is dominant. In a recessive mutation there is still a dominant gene that is healthy. In this case the mutation has no effect on the fitness of the cell. Only when the dominant gene is lost, for example during a chromosome loss, the mutated gene has an effect. This is called loss of heterozygosity [52,53]. In this case, the expression of mutations is dependent on karyotype alterations. In familial cancer types like breast cancer, this mechanism is found: there is a mutation in the BRCA gene in the germline. With the healthy gene from the other parent there are no symptoms. However, when through chromosome loss the dominant healthy gene is lost, a tumour can arise [54,55]. In the research of Pascal Duijf and his colleagues it was found that cancer cells preferentially lose small chromosomes [19]. An example of such a small chromosome is chromosome number 17, the chromosome where the BRCA gene [56] and the P53 gene lie [57]. This chromosome is found to be lost in for example adenocarcinoma [58] and in colorectal tumours [59], suggesting tumours do depend on karyotype alterations for expression via loss of heterozygosity.

Another important fact is that over two third of all tumours are aneuploid [60]. Although this statistic says nothing about cause and consequence, the fact is that a significant amount of tumours are aneuploid, and that is a fact that cannot be dismissed as a coincidence.

Clinical implications

If there is indeed a subpopulation of cells with mutator mutations that can emerge when other cells are destroyed, it is important to also tackle these cells. When these cells are not treated, a whole new tumour with a different phenotype can emerge, which may be more difficult to treat with conventional methods. Although these subpopulations are not yet found, this can be the reason why in some cases after remission the cancer recurs and metastasizes.

If there are indeed cancer specific mutations to be found, these mutations can be a great target for the treatment of tumours. Treatment should be based on the mutations that are found in the tumours. When mutator mutations are found to be the cause of tumours, all tumours can be sequenced to identify the mutation causing that specific tumour. This helps the treatment of tumours with a target gene to attack. If it is true that that these mutator mutations are selected against, this treatment can be combined with usual treatment. The usual treatment eradicates the mass of the tumour, the targeted treatment selectively attacks the subpopulation with the mutator mutation, thus reducing the chance of recurrence after treatment.

Another possibility is to increase the impairment of the DNA repair mechanism. When a cell has too many errors and mutations, even for transformed cells this will be detrimental. If the DNA repair mechanism is already impaired, like in breast cancer with the BRCA1 and BRCA2 gene a little push can drive the cells into apoptosis [61]. Although these drugs will work on all the cells in the body, only cancer cells will be affected because normal cells have a sufficient DNA repair mechanism to survive. Moreover, this plan works perfectly with the current treatment of inducing more DNA damage to the cells. Until now the damage induced can be corrected by the DNA repair mechanism, but when that is impaired the cells can no longer repair and go in apoptosis. Notwithstanding the promising research, caution is necessary, because this treatment has also an effect on unaffected tissues and could cause new mutations and new tumour to arise.

Tumorigenesis by aneuploidy

In a normal human cell, there are 23 chromosome pairs, a total of 46 chromosomes. When a cell replicates and divides, all the replicated chromosomes must be equally divided between the two daughter cells. This process is tightly regulated by a complex mechanism, the cell cycle checkpoints[62]. When the cell cycle checkpoint is impaired, abnormal distribution of the chromosomes can occur, this is a process called chromosomal instability (CIN). This leaves two daughter cells with different chromosome content and these cells are called aneuploid. In 1902 Theodor Boveri had already suggested aneuploidy as a possible cause of cancer [20], but because of the upcoming theory of the somatic mutation, this idea was forgotten for decades. With new techniques it is discovered that two third of cancers are aneuploid [60], therefore aneuploidy is researched again for its carcinogenic function.

In untransformed cells, having abnormal chromosome numbers is detrimental and most of the time not compatible with life. Only in some cases, like trisomy 21 found in Down syndrome, the cells survive during embryonic development. Despite that, with more than 70% of the solid tumours being aneuploid, there seems to be a beneficial effect of aneuploidy [18,19]. The tumorigenesis by aneuploidy theory states that at the origin of cell transformation, a carcinogen produces an aneuploid cell. When this aneuploidy causes for example a third copy of an oncogene, this can drive the formation of a tumour. It is also possible a loss of a chromosome with a tumour suppressor gene can drive tumour formation.

In 2000 Li et al. suggested this model with the following mechanism as depicted in figure 2:

- *Carcinogens induce aneuploidy by chemically or physically altering one or more of the many proteins of the spindle apparatus or the chromosomes, […]. It is also possible that genotoxic carcinogens induce aneuploidy by mutating specific mitosis genes. However, this proposal predicts mutation-specific karyotype instabilities. Instead, the instabilities of the karyotypes of cancer cells, even those from a given clonal cancer, are heterogeneous, but directly proportional to their degree of aneuploidy.*
- *Aneuploidy destabilizes the karyotype and thus initiates an autocatalytic karyotype evolution. This process would generate lethal, preneoplastic and eventually neoplastic karyotypes. The source of the karyotype instability is the imbalance that aneuploidy imparts on the spindle apparatus, e.g., abnormal ratios of spindle proteins and chromosomal proteins and abnormal structures and numbers of centrosomes. The imbalanced spindle will cause chromosome nondisjunction and thus autocatalytically regroup the karyotype, a process that has been termed "chromosome error propagation". The resulting "genetic instability" explains the heterogeneous karyotypes that are a hallmark of cancer. Thus, cancers are clonal for aneuploidy (and certain gene mutations) but not for a particular karyotype.*[21]

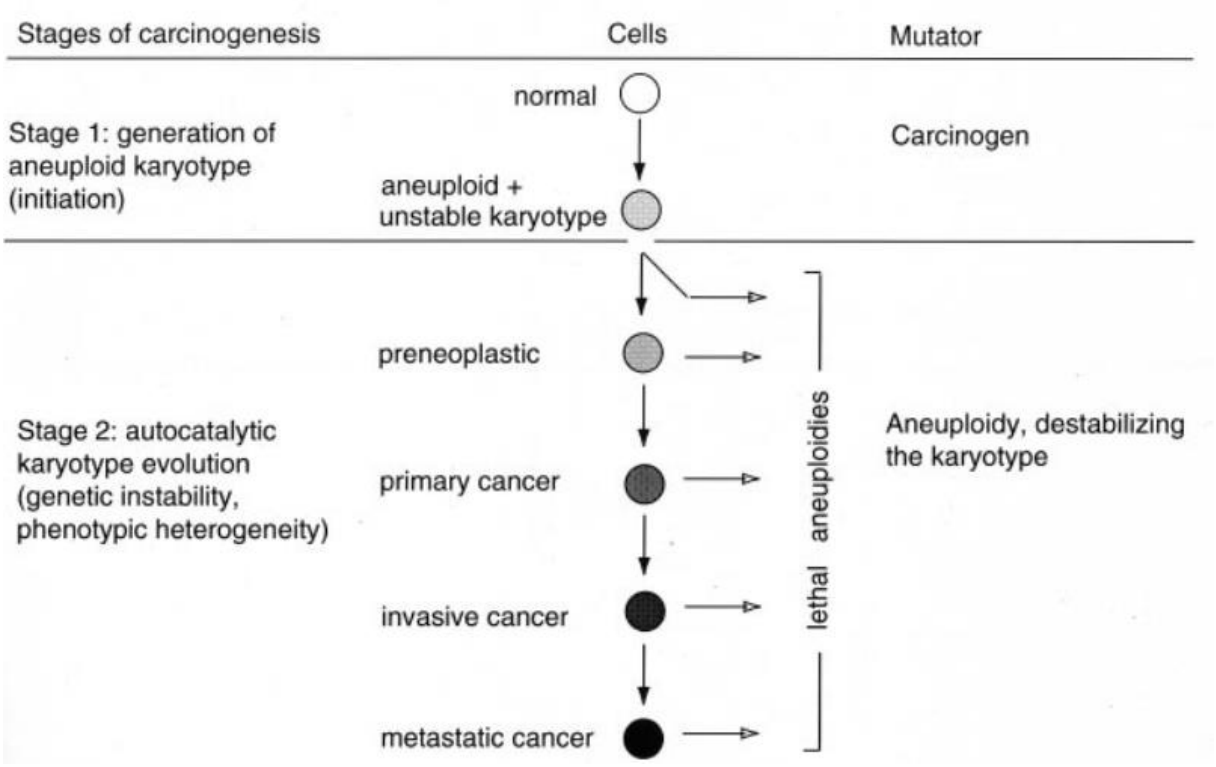

Figure 2. Cascade of aneuploidy during tumorigenesis [21]

This leads to premises that can be experimentally verified:

1. The source of the instability is the imbalance on the spindle apparatus, thus in every cell in tumours there must be some kind of imbalance in the spindle apparatus;
2. Aneuploidy causes transformation, thus experimentally induced aneuploidy must cause tumours;
3. There is a threshold of aneuploidy before transformation;
4. After treatment with a carcinogenic, cells become aneuploid before being transformed;
5. Cancer phenotype is determined by the karyotype.

Experimental evidence

Every of the premises mentioned above will be reviewed and the experimental data will be provided. When there is evidence against this premise it will also be mentioned here.

The source of the instability is the imbalance on the spindle apparatus, thus in every cell in tumours there must be some kind of imbalance in the spindle apparatus.

There are multiple ways in the spindle apparatus to induce CIN and causing aneuploidy, for example a mutation in the spindle assembly checkpoint (SAC). In the research from Daniel Cahill and his colleagues a mutation in the hBUB1 gene was found in colorectal cancer cell lines [63]. Wild type hBUB1 is a mitotic checkpoint gene, and a mutation in this gene allows cells to avoid arresting before dividing even when the chromosomes are not correctly divided, inducing CIN and creating aneuploid daughter cells. Also in lung tumours, in 44% of the tumours an impaired spindle checkpoint was found [64] although the causing mutation is not yet identified. In gastrointestinal cancers not hBUB1 but a mutation in the MAD2 gene caused aneuploidy in cancers. MAD2 is another protein in the spindle

assembly checkpoint [65]. However, in other cancer types, this mutation in the MAD2 gene is not found [66].

Another way the spindle apparatus can affect chromosomal instability is through the transforming acidic coiled-coil (TACC) family. TACC are concentrated at the centrosomes where they regulate microtubule stability [67] and therefore control normal dividing of the chromosomes. Mutations in the TACC gene are found in breast cancer [68], bladder cancer [69] and ovarian cancer [70].

A gene encoding a subunit of the cohesion complex, STAG2, is found to lead to aneuploidy when mutated. This protein regulates the separation of sister chromatids during proliferation. This gene is found to be mutated in diverse human cancers [71]. A mutation here causes the chromatids to separate in a faulty way. Special about this protein that an under expression has this effect, but also over expression. It seems that over expression has an even more significant impact on the prognosis than loss of the protein [72]. This is possible because an under expression only stops the dividing of sister chromatids during dividing, causing the cell to arrest, but over expression could cause the sister chromatids to separate prematurely, causing aneuploidy in the daughter cells.

Despite these results, there is not a single mutation found in all cells of a tumour and there is no evidence that all the cells have an imbalanced spindle apparatus. However, the same method as seen in the model of the single base mutation can be applied here. A high amount of CIN and aneuploidy is detrimental for normal cells and although cancer cells seem to have adapted to these circumstances, a higher amount of aneuploidy could lead to apoptosis, thus leading to selection against imbalance in the spindle apparatus with time. In this case there could also be a quiescent subpopulation with the imbalance of the spindle apparatus, waiting until there is more space to proliferate again. These could well be immune to chemotherapy and therefore important in the treatment of cancer. Another explanation is that mutations are not the only way to cause aneuploidy. As seen in the example of STAG2, an over expression or under expression of proteins can cause aneuploidy. As mentioned earlier, asbestos, hormones, Ni2+ and arsenic induce genomic instability without mutating genes.

Aneuploidy causes transformation, thus experimentally induced aneuploidy must cause tumours.
In mouse models with a Mps1 truncation (a protein in the spindle assemble checkpoint) aneuploidy did not occur spontaneously, however in a p53 deficient setting aneuploidy increased significantly [73]. This can be interpreted as if first a mutation in the p53 gene (a well-known oncogene) must occur before aneuploidy can prevail. One could argue that the p53 deficiency is an environmental factor that is necessary for aneuploidy to prevail. Therefore, aneuploidy is the driving factor and consequently the cause for tumorigenesis and the p53 mutation acts like a carcinogen, indirectly inducing tumorigenesis. Asbestos, as mentioned earlier, is a carcinogenic and induces tumorigenesis by inducing aneuploidy [40]. After the first exposure to asbestos, a tumour arises, indicating aneuploidy is the cause of the formation of the tumour.

There is a threshold of aneuploidy before transformation.
Below the threshold for cancer, cells with aneuploidy only lose small chromosomes and have non cancer phenotypes, like Down's syndrome. Aneuploidy above the threshold involves many or all chromosomes in a cancer cell [21]. The threshold hypothesis helps to explain how it is possible that there is a latent period between exposure with a carcinogenic and the rise of a detectable tumour although this is experimentally not yet verified.

It was however found that patients with aneuploidy breast cancer had a worse prognosis after surgery then patients with a diploid breast tumour [74]. Also the tumours tend to recur more quickly with aneuploidy tumours then with diploid tumours. In colorectal tumours it was found that the higher the aneuploidy the worse the prognosis [75]. The fact that aneuploidy correlates with the prognosis of cancer could imply that more aneuploidy means a more malignant cell. Normal cells that are aneuploidy but are not yet cancer cells are indeed a little malignant. When the malignancy increases in the cells they become cancer cells. However, because there is not yet a definition of this threshold, it is difficult to confirm.

After treatment with a carcinogenic, cells become aneuploid before being transformed.
In 2000 Peter Duesberg and his colleagues performed an experiment in which they induced aneuploidy by exposure to known carcinogenic substances. After the cells became aneuploid, they transformed into cancer cells [76].

In the experiment of Kendall et al. the researchers induced transformation by expressing proteins that inactivate tumour suppressor genes Rb an p53 together with the oncoproteins Ras an Myc and the telomerase subunit hTERT [10]. Two months after transfection with the proteins a tumour arose. The researchers concluded that these few proteins were the only reason for transformation. They made this conclusion based on the fact that two months is too fast for selection or additional events to have happened. This conclusion is questioned in the report of Fabarius et al [77]. The experiment was repeated and karyotypes were analysed. Two months after transfection about half of the cells were aneuploid before transformation. The researchers also found a higher level of dead cells in the culture, indicating a lot of different karyotypes (because not all karyotypes are compatible with survival).

Cancer phenotype is determined by the karyotype.
Aneuploidy is caused by chromosomal instability. Because of this, the chromosomes in all cancer cells are unstable. It is however found that there is an equilibrium between the destabilizing effect of aneuploidy and the stabilizing effect of selection [18]. This means that cancer karyotypes are, in some extent, stable. This correlates with the high level of dead cells that were found in the research of Fabarius et al [77], because only the right karyotypes survive, the others are forced in apoptosis. This was also found in another research, where the karyotypes of cancer cells were somewhat stable [78] and around a small deviation.

The different karyotypes for different tumours also coincides with different phenotypes of cancer. In the research of Andreas Klein in 2010, mammary carcinoma mice cell lines were tested for their karyotype [79]. Different cell lines from different tumours were used, but all the cell lines had the same transgenic oncogenes. All the tumours had different karyotypes, but within the different tumours the karyotypes were significantly alike. It was also seen that different tumours from a common parental carcinoma were closely related. All this evidence suggests that karyotypes in tumours, although aneuploidy is a destabilizing factor, are selected for and are similar in one tumour. They postulate that the mutated oncogenes only indirectly cause cancer, because they destabilize karyotypes, much like carcinogens, and initiate random aneuploidy. Later the researchers made some of the cell lines drug resistant (a new phenotype), and a new karyotype arose. The acquiring of a new phenotype coincided with the development of the new karyotype, therefore it could be concluded that the karyotype determines the phenotype of tumours.

Although aneuploidy is a random process, tumours found in different patients have similar characteristics. This could be explained with convergent evolution. When in evolution a trait appears in two different species without the common ancestor having that trait, this happens through convergent evolution. For example hooves that have evolved from claws several times [80]. Convergent evolution is explained by a similar environment, or in the case of cancer a similar micro-environment. Because of this micro-environment and the selection criteria for the hallmarks of cancer, tumours with similar characteristics evolve even in a random process.

To conclude, although tumours are chromosomal instability and the aneuploidy caused by that is random, within one tumour the karyotype and phenotype is selected for, explaining why the same tumours in different patients have different karyotypes and phenotypes, but one tumour consists of subpopulations of cells with an (almost) alike karyotype.

Clinical implications

When aneuploidy is the cause of cancer, there are other possible treatment options besides targeting mutations. In this model, all the cells have an impaired spindle apparatus. Possible treatment is for example increasing the missegregation rate and therefore increasing the aneuploidy to a point where no cell can survive, and consequently the cells will die [81] .

It is known that in untransformed cells aneuploidy causes a high stress level and cells normally do not survive that stress. Cancers cells have a way to cope with that stress, and even seem to thrive with aneuploidy. Despite that, it is still possible that the transformed cells have more stress than untransformed cells. Increasing this stress can also cause the cell to go into apoptosis. The researchers from lab of Angelika Amon identified compounds increasing this stress and selectively kill aneuploid cells. These compounds are for example AICAR, an energy stress inducer, 17-AAG, an Hsp90 chaperone inhibitor, and chloroquine, an autophagy inhibitor. Although they increase stress in all the cells, untransformed cells survive because they have a lower level of basal stress, while the transformed cells go over a threshold and go in apoptosis [82]. However, these results are not found in other studies with the use of the same compounds. It important to state that the concentration used pushed untransformed cells in apoptosis, therefore could possibly have grave side effects. More research is needed to identify the right concentration and to study the side effects. If the side effects are less severe than with current treatment this could be a great new treatment protocol.

If, on the other hand, not all the cells have an impaired spindle apparatus, but there is a subpopulation of quiescent cells with the impaired spindle apparatus, these cells can survive treatment and cause a recur after treatment. It is important to treat this subpopulation with the right medicines. These cells could possibly be treated with the compounds mentioned earlier, because their basal levels of stress will be higher caused by the impaired spindle apparatus and thus the high amount of chromosomal instability.

Discussion

To answer the question which of the both models explains tumorigenesis and tumour evolution the best, we could use the rule of Occam's razor "*pluralitas non est ponenda sine necessitate"* which translates to "*plurality should not be posited without necessity"* [83], suggesting that between competing theory's, the simpler explanation is preferred . Moreover, this only works when all the evidence can be interpreted both ways. However, with that rule the experimental data gets less significant. To compare the both models, an objective argument must be made for one or the other, but because both models have strong and weak evidence, this is a challenge.

Both models predict some sort of general mutation in all the cells of a tumour, although these are not found yet. Both models also have an acceptable explanation for the lack of these general mutations, and are therefore equal.

Furthermore, both models have an explanation for the heterogeneity found in tumours, and both models also have an explanation for the latent period between mutagenesis and the detection of a tumour. On both these premises the models are equal.

It is proven with experiments that a few mutations can induce a tumour, although these tumours are also found to be aneuploid, suggesting that a combination of both models is the best way to explain tumorigenesis. Aneuploidy is found to be present before transformation to a cancer cell, suggesting aneuploidy is necessary for a cell to transform. These results suggest that both mutations in mutator genes and aneuploidy is necessary before transformation.

A definitive answer to the question which model best explains tumorigenesis and tumour evolution is not possible. Moreover, it seems that both models are a part of the explanation of tumorigenesis and evolution. Therefore, the best way to describe tumorigenesis and tumour evolution, might be a combination of both models.

Proposal new model

In order to treat cancer with a high success rate, it is important to understand the origin of tumour and the mechanisms behind tumour evolution for reasons earlier explained. In this chapter an attempt will be made to combine both models, based on the evidence given in experiments and the premises given by researchers for both models.

Because of the evidence found in experiments, new premises can be formed:

1. Mutator mutations and aneuploidy occur before transformation;
2. A. Mutator mutations can cause aneuploidy or;
   B. Aneuploidy can cause mutator mutations;
3. Microenvironment functions as a bottleneck, explaining the latent period between carcinogenesis and thearise of a detectable tumour;
4. Selection at first drive for more genomic instability, but after time this becomes detrimental and selection reverses, leaving quiescent subpopulations with the detrimental mutations;
5. Tumours are heterogeneous;
6. Convergent evolution causes different tumours to have similar characteristics.

Proposal research

Mutator mutations and aneuploidy occur before transformation.

In new research, the focus should not be on detecting only mutations or aneuploidy, but on a combination of the both: a combination of sequencing and karyotyping before transformation occurs. The research done by Hahn in 1999 could be replicated but with a few changes. In that research hTERT and 2 oncogenes were transfected in cells, but now also a SAC impairment should be introduced. This should reduce the latent period dramatically and could indicate that both a mutator mutation and aneuploidy are necessary for transformation.

Mutator mutations can cause aneuploidy or aneuploidy can cause mutator mutations.

Regular karyotyping after transfection with mutations to confirm aneuploidy before transformation. This indicates that aneuploidy is indeed necessary before transformation. It is already shown in the research of Duesberg and his colleagues that this indeed happens [21].

After inducing aneuploidy, sequencing must be used to confirm other, smaller mutations that occur in mutator genes before transformation. Because aneuploidy cells are inherently genomic unstable, small mutations will occur. Also could it be evolutionary favourable to make small adjustments to the genome when the cell is aneuploid. This could help reducing the level of stress the cell endures.

Microenvironment functions as a bottleneck, explaining the latent period between carcinogenesis and the rise of a detectable tumour.

The amount of nutrition and oxygen for example are selection criteria, this can be confirmed by using medium with less nutrition or incubators with less oxygen. After a latent period of adjusting and selecting the tumour will grow. The transformed cells will have mutations to cope with the new environment. This has influence on the karyotypes and the mutations present, which can both be checked experimentally.

Selection at first drive for more mutations, but after time this becomes detrimental and selection reverses, leaving quiescent subpopulations with the detrimental mutations.

However, after a few passages these mutations will be selected against and lost again. It is therefore essential to do the experiments fast after the initial tumorigenesis. After more passages, subpopulations with these driver mutations will survive and enter a quiescent state. To find these cells, the tumour could be treated. If after a while the tumour recurs, these cells should have the driver mutations again which were also found after the first microenvironment bottlenecks.

Convergent evolution causes different tumours to have similar characteristics.

As explained earlier, different tumours have different karyotypes. However, tumours share similar characteristics, even in different patients. Because these similar characteristics do not come from parentage, they evolved in a similar way. This could be investigated through for example inducing drug resistance. When cell lines are made drug resistant, the phenotype changes and so does the karyotype. The cells get similar characteristics (drug resistance) via different pathways (different cell lines). It must also be examined if also certain mutations are acquired or lost when the cells are made drug resistant.

Although the question of cause and consequence in tumorigenesis is a difficult one, with many possible answers, the new model proposed seems to correspond the most with the data retrieved from experiments. However, although cancer is treated as one disease in research, cancer is a collection of many diseases with similar characteristics. Because of this, all three models could be true for part of

the analysed tumours. With this in mind, it is even more difficult to answer the question which model explains cancer the best. Future research must combine both models to investigate all the possibilities when it comes to tumorigenesis.

Acknowledgements

I would like to thank Bjorn Bakker for his critical reading and encouragements. I would also like to thank the other members of the Foijer Lab, Judith Simon, Klaske Schukken and Petra Bakker for their help and input. I also thank Floris Foijer for generously making the time and space available in his lab while writing this thesis.

Bibliography

1. Cancer Today. Estimated number of deaths, both sexes, all cancers worldwide in 2012. *World Health Organization* (2012). at <http://gco.iarc.fr/today/online-analysis-multi-bars?mode=population&mode_population=continents&population=900&sex=0&cancer=29&type=1&statistic=0&prevalence=0&color_palette=default>
2. Cancer Today. Estimated number of incidence cases, both sexes, all cancers worldwide in 2012. *World Health Organization* (2012). at <http://gco.iarc.fr/today/online-analysis-multi-bars?mode=population&mode_population=continents&population=900&sex=0&cancer=29&type=0&statistic=0&prevalence=0&color_palette=default>
3. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: The next generation. *Cell* **144,** 646–674 (2011).
4. Tyzzer, E. Tumor Immunity. *J. Cancer Res.* **1,** 125–156 (1916).
5. Berenblum, I. & Shubik, P. An experimental study of the initiating state of carcinogenesis, and a re-examination of the somatic cell mutation theory of cancer. *Br. J. Cancer* **3,** 109–118 (1949).
6. Auerbach, C. & Robson, J. M. Chemical production of mutations. *Nature* **157,** 302 (1946).
7. Armitage, P; Doll, R. The Age Distribution of Cancer and a Multi-Stage Theory of Carcinogenesis. *Br. J. Cancer* **8,** 1–12 (1954).
8. Vogelstein, B. & Kinzler, K. W. The multistep nature of cancer. *Trends Genet.* **9,** 138–141 (1993).
9. Hahn, W. C. *et al.* Creation of human tumour cells with defined genetic elements. *Nature* **400,** 464–8 (1999).
10. Kendall, S. D., Linardic, C. M., Adam, S. J. & Counter, C. M. A network of genetic events sufficient to convert normal human cells to a tumorigenic state. *Cancer Res.* **65,** 9824–9828 (2005).
11. Salk, J. J., Fox, E. J. & Loeb, L. a. Mutational heterogeneity in human cancers: origin and consequences. *Annu. Rev. Pathol.* 51–75 (2010). doi:10.1146/annurev-pathol-121808-102113.Mutational
12. Bakker, B. *et al.* Single cell sequencing reveals karyotype heterogeneity in murine and human tumours. *Genome Biol.* 1–15 (2016). doi:10.1186/s13059-016-0971-7
13. Swanton, C. Intratumor heterogeneity: Evolution through space and time. *Cancer Res.* **72,** 4875–4882 (2012).
14. Vogelstein, B. *et al.* Cancer Genome Landscapes. *Science (80-.).* **339,** 1546–1558 (2013).
15. Loeb, L. A., Loeb, K. R. & Anderson, J. P. Multiple mutations and cancer. *Proc. Natl. Acad. Sci. U. S. A.* **100,** 776–81 (2003).
16. Loeb, L. A. Human cancers express mutator phenotypes: origin, consequences and targeting. *Nat. Rev. cancer* **11,** 450–457 (2011).
17. Gordon, D. J., Resio, B. & Pellman, D. Causes and consequences of aneuploidy in cancer. *Nat. Rev. Genet.* **13,** 189–203 (2012).
18. Li, L. *et al.* Cancer-causing karyotypes: chromosomal equilibria between destabilizing aneuploidy and stabilizing selection for oncogenic function. *Cancer Genet. Cytogenet.* **188,** 1–25 (2009).
19. Duijf, P. H., Schultz, N. & Benezra, R. Cancer cells preferentially lose small chromosomes. *Int J*

Cancer **132,** 2316–2326 (2013).

20. Boveri, T. Uber mehrpolige mitosen als mittel zur analyse des zellekrns. *Verhandlungen der Phys. Gesellschaft zu Würzbg.* **35,** 67–90 (1902).
21. Li, R., Sonik, A., Stindl, R., Rasnick, D. & Duesberg, P. Aneuploidy vs. gene mutation hypothesis of cancer: recent study claims mutation but is found to support aneuploidy. *PNAS* **97,** 3236–3241 (2000).
22. Ames, B. N. Identifying Environmental Chemicals Causing Mutations and Cancer. *Science (80-.).* **204,** 587–593 (1979).
23. Ferlay, J. *et al.* GLOBOCAN 2012: Estimated Cancer Incidence, Mortailty and Prevalence Worldwide in 2012. *Non-communicable disease factsheet. [Online].* (2013). at <http://globocan.iarc.fr/Pages/fact_sheets_population.aspx>
24. J, F. *et al.* incidence top 20 cancers worldwide 2012. *World Cancer Research Fund International* (2014). at <http://www.wcrf.org/int/cancer-facts-figures/worldwide-data>
25. Klein, C. A. The Metastasis Cascade. *Science (80-.).* **321,** 1785–1787 (2008).
26. Hall, B. G. Spontaneous point mutations that occur more often when advantageous than when neutral. *Genetics* **126,** 5–16 (1990).
27. Schmitt, M. W., Prindle, M. J. & Loeb, L. A. Implications of genetic heterogeneity in cancer. *Ann N Y Acad Sci.* **September,** 110–116 (2012).
28. Loeb, L. a, Springga.Cf & Battula, N. Errors in DNA-replication as a basis of malignant changes. *Cancer Res.* **34,** 2311–2321 (1974).
29. Kolodner, R. D., Putnam, C. D. & Myung, K. Maintenance of genome stability in Saccharomyces cerevisiae. *Science* **297,** 552–7 (2002).
30. Smith, J., Mun Tho, L., Xu, N. & A. Gillespie, D. *The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer*. *Advances in Cancer Research* **108,** (Elsevier Inc., 2010).
31. Rayner, E. *et al.* A panoply of errors: polymerase proofreading domain mutations in cancer. *Nat. Rev. Cancer* **16,** 71–81 (2016).
32. Kunkel, T. a & Bebenek, K. DNA Replication Fidelity. *Environ. Heal.* 497–529 (2000). doi:10.1146/annurev.biophys.34.040204.144537
33. Palles, C. *et al.* Germline mutations in the proof-reading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat. Genet.* **45,** 136–144 (2013).
34. Yoshida, K. & Miki, Y. Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Sci.* **95,** 866–871 (2004).
35. Deng, C.-X. Roles of BRCA1 in centrosome duplication. *Oncogene* **21,** 6222–6227 (2002).
36. Cortez, D. Requirement of ATM-Dependent Phosphorylation of Brca1 in the DNA Damage Response to Double-Strand Breaks. *Science (80-.).* **286,** 1162–1166 (1999).
37. Easton, D. F., Ford, D. & Bishop, D. T. Breast and ovarian cancer incidence in BRCA1-mutation carriers. *Am. J. Hum. Genet.* **56,** 265–271 (1995).
38. Rosen, E. M., Fan, S., Pestell, R. G. & Goldberg, I. D. BRCA1 gene in breast cancer. *J. Cell. Physiol.* **196,** 19–41 (2003).
39. Jordan, C. T., Guzman, M. L. & Noble, M. Cancer Stem Cells. *N. Engl. J. Med.* **355,** 1253–1261 (2006).
40. Barrett, J. C., Lamb, P. W. & Wiseman, R. W. Multiple mechanisms for the carcinogenic effects of asbestos and other mineral fibers. *Environ. Health Perspect.* **81,** 81–89 (1989).

41. Waldum, H. L., Brenna, E., Sandvik, A. K., Syversen, U. & Falkmer, S. Hormones and carcinogenesis. *Endocr. Relat. Cancer* **5,** 45–48 (1998).

42. Rossman, T. G. Mechanism of arsenic carcinogenesis: An integrated approach. *Mutat. Res. - Fundam. Mol. Mech. Mutagen.* **533,** 37–65 (2003).

43. Sunderman, F. W. Mechanisms of nickel carcinogenesis. *Scand. J. Work. Environ. Heal.* **15,** 1–12 (1989).

44. Lijinsky, W. A View of the Relation Between Carcinogenesis and Mutagenesis. *Evironmental Mol. Mutagen.* **14,** 78–84 (1989).

45. Struewing, J. P. *et al.* The Risk of Cancer Associated With Specific Mutations. *N. Engl. J. Med.* **336,** 1401–1408 (1997).

46. Surget, S., Khoury, M. P. & Bourdon, J. C. Uncovering the role of p53 splice variants in human malignancy: A clinical perspective. *Onco. Targets. Ther.* **7,** 57–67 (2013).

47. Soussi, T., Ishioka, C., Claustres, M. & Béroud, C. Locus-specific mutation databases: pitfalls and good practice based on the p53 experience. *Nat. Rev. Cancer* **6,** 83–90 (2006).

48. Shih, I. *et al.* Evidence That Genetic Instability Occurs at an Early Stage of Colorectal Tumorigenesis Advances in Brief Evidence That Genetic Instability Occurs at an Early Stage of Tumorigenesis. *Oncology* 818–822 (2001).

49. Willenbucher, R. F. *et al.* Genomic instability is an early event during the progression pathway of ulcerative-colitis-related neoplasia. *Am. J. Pathol.* **154,** 1825–30 (1999).

50. Niu, Y., Wang, Y. & Zhou, D. The phenotypic equilibrium of cancer cells: From average-level stability to path-wise convergence. *J. Theor. Biol.* **386,** 7–17 (2015).

51. Gupta, P. B. *et al.* Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. *Cell* **146,** 633–644 (2011).

52. Deng, G., Lu, Y., Zlotnikof, G., Thor, A. D. & Smith, H. S. Loss of heterozygosity in normal tissue adjacent to breast carcinomas.pdf. *Science (80-.).* **274,** 2057–2059 (1996).

53. Allendorf, F. W. Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biol.* **5,** 181–190 (1986).

54. Cornelisse, C. J., Kuipers-dijkshoorn, N., Vliet, M. van, Hermans, J. & Devilee, P. Fractional allelic imbalance in human breast cancer increases with tetraploidization and chromosome loss. *Int. J. Cancer* **548,** 544–548 (1992).

55. Yee, C. J., Rood, N., Verrier, C. S. & Pari, F. F. Microsatellite Instability and Loss of Heterozygosity in Breast Cancer. *Cancer* **54,** 1641–1644 (1994).

56. Home, R. G. BRCA gene. *U.S. National library of medicine* (2016). at <https://ghr.nlm.nih.gov/gene/BRCA1>

57. Isobe, M., Emanuel, B. S., Givol, D., Oren, M. & Croce, C. M. Localization of gene for human p53 tumour antigen to band 17p13. *Nature* **320,** 84–85 (1986).

58. Swift, A. *et al.* Frequent loss of heterozygosity on chromosome 17 at 17q11.2-q12 in Barrett's adenocarcinoma. *Br. J. Cancer* **71,** 995–998 (1995).

59. Yamamoto, L., Lopes, A. A., Harb-Gama, A. & Nagai, M. A. TP53 mutations and loss of heterozygosity of chromosome 17 in colorectal tumors. *Brazilian J. Genet.* **19,** 647–653 (1996).

60. Weaver, B. a. & Cleveland, D. W. Does aneuploidy cause cancer? *Curr. Opin. Cell Biol.* **18,** 658–667 (2006).

61. Helleday, T., Petermann, E., Lundin, C., Hodgson, B. & Sharma, R. A. DNA repair pathways as

targets for cancer therapy. *Nat Rev Cancer* **8,** 193–204 (2008).

62. Cooper, G. M. *The cell: a molecular approach. The eukaryotic cell cycle*. (Sinauer Associates, 2000). doi:10.1016/S0300-483X(02)00460-2

63. Cahill, D. P. *et al.* Mutations of mitotic checkpoint genes in human cancers. *Lett. to Nat.* **392,** 300–303 (1998).

64. Takahashi, T. *et al.* Identification of frequent impairment of the mitotic checkpoint and molecular analysis of the mitotic checkpoint genes, hsMAD2 and p55CDC, in human lung cancers. *Oncogene* **18,** 4295–4300 (1999).

65. Kim, H. S. *et al.* Frequent mutations of human Mad2, but not Bub1, in gastric cancers cause defective mitotic spindle checkpoint. *Mutat. Res. - Fundam. Mol. Mech. Mutagen.* **578,** 187–201 (2005).

66. Ruddy, D. A., Gorbatcheva, B., Yarbrough, G., Schlegel, R. & Monahan, J. E. No somatic mutations detected in the Mad2 gene in 658 human tumors. *Mutat. Res. - Fundam. Mol. Mech. Mutagen.* **641,** 61–63 (2008).

67. Raff, J. W. Centrosomes and cancer: Lessons from a TACC. *Trends Cell Biol.* **12,** 222–225 (2002).

68. Cully, M. *et al.* Transforming acidic coiled coil 1 promotes transformation and mammary tumorigenesis. *Cancer Res.* **65,** 10363–10370 (2005).

69. Kiemeney, L. a. L. a L. a. *et al.* A sequence variant at 4p16. 3 confers susceptibility to urinary bladder cancer. *Nat. Genet.* **42,** 415–419 (2010).

70. Lauffart, B. *et al.* Aberrations of TACC1 and TACC3 are associated with ovarian cancer. *BMC Womens. Health* **5,** 8 (2005).

71. Solomon, D. A. *et al.* Mutational Inactivation of STAG2 Causes Aneuploidy in Human Cancer. *Science (80-.).* **333,** 1039–1043 (2012).

72. Rhodes, J. M., McEwan, M. & Horsfield, J. a. Gene Regulation by Cohesin in Cancer: Is the Ring an Unexpected Party to Proliferation? *Mol. Cancer Res.* **9,** 1587–1607 (2011).

73. Foijer, F. *et al.* Chromosome instability induced by Mps1 and p53 mutation generates aggressive lymphomas exhibiting aneuploidy-induced stress. *Proc. Natl. Acad. Sci. U. S. A.* **111,** 13427–32 (2014).

74. Owainati, a a *et al.* Tumour aneuploidy, prognostic parameters and survival in primary breast cancer. *Br. J. Cancer* **55,** 449–454 (1987).

75. Risques, R. *et al.* Redefining the significance of aneuploidy in the prognostic assessment of colorectal cancer. *Labaratory Investig.* **81,** 3074–315 (2001).

76. Duesberg, P. *et al.* Aneuploidy precedes and segregates with chemical carcinogenesis. *Cancer Genet. Cytogenet.* **119,** 83–93 (2000).

77. Fabarius, A., Li, R., Yerganian, G., Hehlmann, R. & Duesberg, P. Specific clones of spontaneously evolving karyotypes generate individuality of cancers. *Cancer Genet. Cytogenet.* **180,** 89–99 (2008).

78. Nicholson, J. M. & Duesberg, P. On the karyotypic origin and evolution of cancer cells. *Cancer Genet. Cytogenet.* **194,** 96–110 (2009).

79. Klein, A. *et al.* Transgenic oncogenes induce oncogene-independent cancers with individual karyotypes and phenotypes. *Cancer Genet. Cytogenet.* **200,** 79–99 (2010).

80. Prothero, D. R., Manning, E. M. & Fischer, M. The phylogeny of the ungulates. *Phylogeny Classif. Tetrapods* **2,** 201–234 (1988).

81. Kops, G. J. P. L., Weaver, B. a a & Cleveland, D. W. On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat. Rev. Cancer* **5,** 773–85 (2005).

82. Tang, Y. C., Williams, B. R., Siegel, J. J. & Amon, A. Identification of aneuploidy-selective antiproliferation compounds. *Cell* **144,** 499–512 (2011).

83. Duignan, B. Occam's razor. *Encyclopaedia Brittanica* (2015). at <https://www.britannica.com/topic/Occams-razor>