Chromosomal Instability

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Chromosomal instability is the process of mis-segregation for ongoing chromosomes, which leads to cells with an abnormal number of chromosomes, also known as an aneuploid state. Induced aneuploidy is detrimental during development and in primary cells but aneuploidy is also a hallmark of cancer cells. It is therefore believed that premalignant cells need to overcome aneuploidy-imposed stresses to become tumorigenic. Over the past decade, some aneuploidy-tolerating pathways have been identified through small-scale screens, which suggests that aneuploidy tolerance pathways can potentially be therapeutically exploited. However, to better understand the processes that lead to aneuploidy tolerance in cancer cells, large-scale and unbiased genetic screens are needed, both in euploid and aneuploid cancer models.

Keywords: chromosomal instability ; genome wide screens ; cancer

1. Introduction

During each cell division, a cell's genome is replicated, after which all chromosomes need to be properly distributed over the two emerging daughter cells. Continuous errors during chromosome segregation, also known as chromosomal instability (CIN), leads to cells with chromosome numbers that deviate from the euploid karyotype, a state defined as aneuploid [1]. Aneuploidy is highly detrimental during development, which is reflected by the fact that it is the leading cause of spontaneous abortion and mental retardation in humans [2]. When induced experimentally, aneuploidy negatively affects cellular fitness by reducing cell growth and inducing metabolic and proteotoxic stress [3][4][5][6]. However, aneuploidy is a hallmark of cancer [7][8], a disease characterized by uncontrolled proliferation. This apparent contraction, also known as the aneuploidy paradox [9], suggests that aneuploid cells must activate 'aneuploidy-coping' mechanisms in order to adopt a malignant fate. Therefore, the cellular stresses imposed by aneuploidy are considered to be attractive targets for therapeutic intervention.

The currently-known aneuploidy-tolerating hits and pathways have mostly been identified from small scale screens or through educated guesses using model systems for aneuploid non-transformed cells or cancer cell lines. While these findings are key for our understanding of the biology of aneuploid cells, they unlikely draw the complete picture of aneuploidy tolerance pathways. This is partly because the screens and model systems used are biased towards pathways that we already understand reasonably well. Furthermore, these experiments were mostly done in cultured cells and thus do not account for the in vivo malignant transformation process and interactions between tissues. To acquire a more comprehensive overview of how cells adapt to aneuploidy during malignant transformation, unbiased genome-wide in vivo screens that carefully compare the tumor drivers between aneuploid and euploid cancers are a next important step forward.

2. How Genetic Mutations Collaborate with Genomic Instability in Cancer

Aneuploidy is mostly detrimental for cells and initially leads to a proliferative disadvantage, presumably due to the activation of aneuploidy-imposed stress pathways. It is therefore likely that aneuploid cells, throughout their malignant transformation process, need to overcome these stresses. Therefore, the molecular mechanisms underpinning these aneuploidy-induced stresses are considered to be promising therapeutic targets. The work of many labs in the last 15 years has significantly improved our understanding of some of the roadblocks that aneuploid cells need to overcome during tumorigenesis. However, to our knowledge, no large-scale screens have been reported that systematically compare the pathways affected in aneuploid cancers to the those affected in euploid cancers. When performed in an isogenic setting, such screens would surely reveal the differences between euploid and aneuploid cells on their route to a malignant program.

Five types of mutagenesis screens that could be suitable for this goal were discussed, each with their own advantages and disadvantages (Table 1). ENU mutagenesis could be very effective in screening for point mutations that would accelerate the transformation of aneuploid cells. However, identifying the individual mutations that drive the phenotype is extremely laborious and many mice would be needed when such a screen would be performed in vivo. Retroviral mutagenesis allows for rapid identification of the mutated gene that improves the survival of aneuploid cells. However, these screens only sample proliferative tissues as the virus will only integrate in dividing cells. Because of this important limitation, retroviral tagging screens have mostly been surpassed by transposon, RNAi, and CRISPR screens. Indeed, transposon mutagenesis can be induced in any cell type within the whole organism, using a ubiquitously expressed transposase or in individual tissues with a conditional transposase controlled by a tissue-specific Cre-recombinase. Transposon mutagenesis furthermore allows for the identification of multiple collaborating driver mutations, which more accurately reflects the complexity of human cancer than a single mutation. However, transposons do display some insertion site preference, which yields to some bias in the screened part of the genome. This problem was largely overcome with the introduction of PiggyBac transposons, which suffer less from 'local hopping' and thus target the whole genome more efficiently [77]. In CRISPR/Cas9 and RNAi interference screens, such bias can be eliminated by careful sgRNA/shRNA/RNAi library design. RNAi have lost some popularity at the benefit of CRISPR screens, as CRISPR screens completely inactivate the targeted genes instead of (partially) knocking gene expression down and display fewer off-target effects. Moreover, CRISPR genome engineering offers many more applications, such as knockdown, knockout, knock-in, activation, and base editing [10][11], all of which can be exploited in genetic screens.

Table 1. The advantages and disadvantages of several mutagenesis systems.

Mutagenesis System	Advantages	Disadvantages
Chemical	• Induces point mutations • Unbiased disease gene discovery based on phenotyping • Can be used in forward and reverse genetic approaches • In vitro and in vivo use	• Labor intensive positional cloning to identify mutated gene • Identification of recessive genes in vivo requires back- or inter-crossing; many mice required • Base pair substitution bias; some genes or domains more frequently mutated
Retrovirus	• Rapid identification of mutated gene • Does not require generation of transgenic mice for in vivo screens • In vitro and in vivo use	• Mostly identifies gain of function mutations • (Most) cells must be dividing for retrovirus integration • Strain-specific effects and limitations • Limited tissue flexibility
Transposon	• Genome-wide • Loss and gain of function • In vitro and in vivo use • Allows for the identification of multiple cooperating mutations • Can identify the effects of mutations in non-coding regions of the genome • Can be done in vivo in whole organism or in tissue specific setup	• Requires generation of transgenic lines • Insertion site preference leading to bias • SB has tendency for local hopping, and leaves footprint behind. Note that these disadvantages are not true for PB transposons • Does not allow for identification of point mutations

Mutagenesis System	Advantages	Disadvantages
RNA interference	• Genome-wide • Stable • In vitro and in vivo use	• Only loss of function • Off target effects • Does not identify multiple cooperating genetic mutations required for phenotype
CRISPR-Cas9	• Genome-wide • Can identify loss and gain of function mutations (CRISPRi/CRISPRa) • In vitro and in vivo use • Can be done in vivo in whole organism or in tissue specific setup	• Does not identify multiple cooperating genetic mutations required for phenotype

Altogether, to identify in an unbiased fashion the changes needed to convert an aneuploid cell into a cancer cell, one would need to setup an in vivo screen that would compare tumorigenesis in an euploid and aneuploid background. As stable aneuploidy is probably not sufficient to accelerate cancer in mice, the aneuploid background would need to be generated by crossing the 'screening mice' into a well-characterized CIN-predisposed background. This would likely work well as in many mouse models for CIN-driven cancer, CIN alone is not a powerful driver of cancer, but rather an accelerator [12][13]. Given that ongoing CIN is incompatible with early embryonic development [12], the most suitable CIN predisposition would be a conditional CIN-driving allele that does not efficiently promote cancer by itself. This could for instance be a Mps1 truncation or mutation allele [14][15], a Mad2 deletion allele [16], a hypomorphic BubR1 allele [17], or a Plk4 overexpression allele [18], as well as any other tissue-specific CIN driver. Indeed, in most of the CIN models, the CIN-driving allele alone leads to aneuploidy but not to rapid tumorigenesis. However, combining CIN with a single mutation in p53 not only leads to cancer initiation [14][16][19][15][17][20] but also to a significant reduction of tumor latency, which makes this setup very suitable for a mutagenesis screen.

Altogether, we conclude that genome-wide mutagenesis screens in a CIN-predisposed background will likely yield important steps forward in the identification of more mechanisms of aneuploidy tolerance in vivo.

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