Aneuploidy: Cancer strength or vulnerability?

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Aneuploidy is a very rare and tissue-specific event in normal conditions, occurring in a low number of brain and liver cells. Its frequency increases in age-related disorders and is one of the hallmarks of cancer. Aneuploidy has been associated with defects in the spindle assembly checkpoint (SAC). However, the relationship between chromosome number alterations, SAC genes and tumor susceptibility remains unclear. Here, we provide a comprehensive review of SAC gene alterations at genomic and transcriptional level across human cancers and discuss the oncogenic and tumor suppressor functions of aneuploidy. SAC genes are rarely mutated but frequently overexpressed, with a negative prognostic impact on different tumor types. Both increased and decreased SAC gene expression show oncogenic potential in mice. SAC gene upregulation may drive aneuploidy and tumorigenesis through mitotic delay, coupled with additional oncogenic functions outside mitosis. The genomic background and environmental conditions influence the fate of aneuploid cells. Aneuploidy reduces cellular fitness. It induces growth and contact inhibition, mitotic and proteotoxic stress, cell senescence and production of reactive oxygen species. However, aneuploidy confers an evolutionary flexibility by favoring genome and chromosome instability (CIN), cellular adaptation, stem cell-like properties and immune escape. These properties represent the driving force of aneuploid cancers, especially under conditions of stress and pharmacological pressure, and are currently under investigation as potential therapeutic targets. Indeed, promising results have been obtained from synthetic lethal combinations exploiting CIN, mitotic defects, and aneuploidy-tolerating mechanisms as cancer vulnerability.

Aneuploidy: A Normal and Abnormal Condition

Normal human diploid cells contain 23 pairs of chromosome (44 autosomes and two sex chromosomes). In some circumstances, the number of whole chromosomes is altered, a condition known as aneuploidy. Aneuploidy is physiological during cellular development in some tissues (e.g., in liver and...
brain), probably because of its contribution to cellular diversity, which provides a selective advantage in response to injuries. Binucleated and polyploid hepatocytes are detectable in mice few weeks after birth. They can revert to diploidy and become aneuploid, while diploid liver cells can increase their ploidy. This dynamic mechanism, defined as “ploidy-conveyor,” generates aneuploidy and has also been reported in human hepatocytes. Under conditions of stress, hepatocytes acquire specific aneuploidies enabling them to resist to chronic liver injuries. Moreover, aneuploid neurons, retaining functional activity, have been observed in developing and adult murine models and in the human brain. The majority of studies have reported a prevalence of aneuploid cells exceeding 50% and 20% in the liver and brain, respectively. However, SKY and FISH approaches, which have been used for karyotype analysis, may overestimate aneuploidy. Indeed, a recent single cell sequencing study revealed that even in liver and brain tissues, aneuploidy accounted for less than 5% of all cells under physiological conditions. In different tissues, aneuploidy is associated with aging and age-related disorders. Mice expressing reduced levels of the spindle assembly checkpoint (SAC) component BUBR1, which is mutated in the majority of patients with Mosaic Variegated Aneuploidy (MVA) syndrome, develop progressive aneuploidy and age-related defects including cataracts, loss of subcutaneous fat, skeletal muscle wasting, lordokyphosis, impaired wound healing and cerebral degeneration, with deficits in neural progenitor proliferation and maturation. In oocytes, the frequency of chromosome segregation errors in meiosis I increase with maternal aging, along with a decrease in BUBR1 protein. The aneuploid condition may also favor neurodegeneration during aging. The APP gene, which encodes for the protein forming amyloid β plaques in Alzheimer’s disease, is located on chromosome 21. Individuals with Down’s syndrome frequently develop this neurodegenerative disorder by the age of 40, and buccal cells from patients with Alzheimer’s disease frequently carry trisomy of chromosomes 21 or 17, where many susceptibility genes are located. These findings suggest that a low frequency of aneuploid cells can be tolerated or may even be advantageous under specific conditions in nonmalignant tissues, whereas increased rates of aneuploidy can become pathogenic, as observed in neurodegenerative diseases and in cancer. Theodor Boveri initially suggested that an abnormal chromosome number causes tumorigenesis. Over the past 100 years, a number of studies have investigated the cellular and molecular events that cause aneuploidy and studied its potential involvement in cancer development. Here, we describe SAC gene alterations across tumors and their link with neoplastic transformation. We also focus on the complex relationship between aneuploidy and cancer, including the oncogenic and tumor suppressor functions of the abnormal chromosome number and its therapeutic potential.

The Spindle Assembly Checkpoint in Aneuploidy Generation and Cancer
Aneuploidy in mitotically dividing cells can result from numerous defects, including mitotic slippage, cytokinesis failure, spindle multipolarity, defective kinetochore-microtubule attachments, perturbed microtubule dynamics, cohesion defects, and impaired SAC function. The SAC prevents entry into anaphase and premature chromosome segregation until all kinetochores are properly attached to the mitotic spindle. This function is achieved through assembly of the mitotic checkpoint complex (MCC), the SAC effector, which inhibits the activity of the anaphase-promoting complex/cyclosome (APC/C)\(^{1,27}\). Briefly, when the SAC is satisfied, the MCC is disassembled and APC/C\(^{CdC20}\) drives ubiquitination and proteolytic degradation of cyclin B1 and securin. These events induce mitotic exit and sister chromatid separation by degradation of the cohesin complex. A weakened SAC may allow cells to enter anaphase in the presence of unattached or misaligned chromosomes and both copies of one chromosome may be deposited into a single daughter cell (Fig. 1). Thus, failure of the SAC machinery is an obvious candidate mechanism involved in the generation of aneuploidy during mitosis. However, the genes encoding SAC proteins (including MAD1L1, BUB1, BUB1B, CDC20, BUB3, and MAD2L1) are rarely mutated in human cancers. A mutation frequency exceeding 5% has been only detected in uterine corpus endometrial carcinoma (9.6, 7.4, 6.2, 6.4, and 7.6% of patients carrying MAD1L1, BUB1, BUB1B, CDC20, or BUB3 mutations, respectively) and colon adenocarcinoma (5.5% of patients with MAD1L1 or BUB1 mutations) according to next generation sequencing data from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/). On the contrary, SAC genes are deregulated at mRNA and protein level in a number of tumors (Table 1), suggesting potential alterations of epigenetic, transcriptional and post-transcriptional regulation. For example, mutations of oncogenic or tumor suppressor pathways can lead to deregulated SAC gene expression. There is evidence that inactivating RB mutations cause deregulation of the E2F family of transcription factors resulting in MAD2 overexpression and chromosome instability (CIN), which have also been detected in a p53 mutant mouse model. With the exception of a few reported cases of reduced expression, SAC genes are generally overexpressed in primary tumors (Table 1). High expression levels associate with elevated proliferation index and metastatic potential and predict advanced stage, reduced overall survival, disease-free survival and recurrence-free survival across several cancer types, including solid tumors, and hematological malignancies. This observation appears in contrast to the fact that aneuploidy occurs in cases of defective SAC. However, both increased and decreased SAC gene expression induces aneuploidy and favors tumor development, as demonstrated in mice (Table 2). The protumorigenic or antitumorigenic effect is also dependent on the specific SAC gene which is

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Simonetti et al. (2019) - Int. J. Cancer: 144, 8–25 (2019) © 2018 The Authors. International Journal of Cancer published by John Wiley & Sons Ltd on behalf of UICC.
overexpressed or downregulated. For example, CDC20 overexpression impairs SAC function and favors aneuploidization in oral cancer. Moreover, chromosome missegregation and aneuploidy have been reported in both transgenic (tg), hypomorphic and haploinsufficient mouse models, including MAD2-tg and mad2\(^{+/−}\), BUB1-tg, bub1\(^{Δ2–3}/Δ2–3\), bub1\(^{−/−}\) and bub1\(^{+/−}\). These findings suggest that expression of some SAC genes above threshold levels is required to maintain genomic stability and prevent tumorigenesis, as shown in the bub1\(^{+/−}\) model, characterized by higher levels of BUB1 protein compared to bub1\(^{−/−}\) and bub1\(^{+/−}\) mice and lower tumor incidence. However, SAC gene deficiency is also detrimental, probably due to extreme CIN. Indeed homozygous deletion of many SAC components causes early embryonic lethality. Conversely, the protumorigenic effect of SAC gene overexpression may be linked to delayed mitotic exit, which induces aneuploidy (e.g., MAD2 overexpression stabilizes securin and cyclin B and inhibits cytokinesis), and to potential oncogenic roles of SAC proteins outside mitosis: BUBR1 plays a role in DNA damage responses, CDC20 is involved in the regulation of apoptosis, DNA repair and stem-like cell properties and MAD1 has a function at the Golgi apparatus. This complex scenario suggests that both increased and decreased SAC gene expression may favor tumorigenesis, depending on the threshold level, the gene functions inside and outside mitosis, the effect on chromosome stability, the cell type and its genomic background.

**Tumor-Protecting and Tumor-Promoting Effects of Aneuploidy**

The complex and much debated relationship between aneuploidy and cancer fosters a very active research field. There is evidence to suggest that aneuploidy can exert an antitumorigenic or a protumorigenic effect (Fig. 3). Studies on yeast strains, murine and human cells have shown that aneuploidy impairs the proliferative capacity of nonmalignant cells and that the phenotype is independent of the identity of the individual chromosomes, while being potentially proportional to its size. Changes in chromosome copy number result in transcriptomic alterations and gene-dosage effects at the proteomic level, as extensively reviewed by Ried et al. These in turn lead to an imbalance in the cellular protein composition, which may saturate the protein folding and degradation machineries, thus eliciting a proteotoxic stress response and altering the redox anabolic homeostasis, leading to increased reactive oxygen species (ROS). These findings indicate that aneuploidy is generally disadvantageous for cells and there is ample evidence of the negative effect of aneuploidy on the fitness of nonmalignant cells (Fig. 3a). First, aneuploidy is an extremely rare event in normal conditions, even in brain and liver. Second, trisomic murine embryonic fibroblasts (MEFs) show contact inhibition properties, proliferation arrest in low...
serum medium, lack of clonogenic capacity and senescence features after 7–10 passages in culture.\textsuperscript{52} Third, trisomic cells can revert to the euploid state by losing extra chromosomes both in vitro and in vivo in order to improve their growth capacity.\textsuperscript{52} Accordingly, human fibroblasts from patients with constitutional triploidy show moderate levels of somatic mosaicism due to the progressive accumulation of cells that undergo whole chromosome loss.\textsuperscript{53} Moreover, constitutional aneuploidy per se is not sufficient to generate tumor-like CIN.\textsuperscript{53} Aneuploid cells modulate their metabolic and transcriptional programs to improve their fitness. Indeed, aneuploidy is associated with higher glucose and/or glutamine consumption\textsuperscript{46,48} and with changes in the expression of proteins involved in cell cycle, ribosome biogenesis, endoplasmic reticulum, Golgi apparatus, lysosomes, membrane metabolism, the major histocompatibility complex (MHC) protein complex and antigen processing, DNA replication, transcription, energy production, and response to stress.\textsuperscript{44,45,54} These pathways are deregulated in several aneuploid cell lines, although the specific combinations of genes displaying altered expression differ.\textsuperscript{51}

These observations, mostly obtained from trisomic models, indicate that single-chromosome aneuploidy is not sufficient per se to induce malignant transformation\textsuperscript{52} but rather has antitumorigenic properties. Accordingly, individuals with Down’s syndrome display a reduced incidence of solid tumors, including breast, lung, and prostate cancers, suggesting that trisomy 21 is a protective event against malignant transformation in those tissues.\textsuperscript{55} Moreover, some mouse models with deregulated SAC genes that develop aneuploidy have a decreased rate of tumorigenesis (Table 2), even under

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**Figure 3.** The complex relationship between aneuploidy and cancer. (a) Aneuploidy-related growth and contact inhibition, ROS production, cell senescence can cooperate with environmental conditions and tumor suppressor activity to inhibit malignant transformation (round shaped cells represent nontransformed cells). (b) When prosurvival and protumorigenic events induced by aneuploidy (anchorage-independent growth, transcriptional and metabolic reprogramming, GIN, CIN, immune escape) synergize with activation of oncogenes and favorable environmental conditions, cells carrying an aberrant chromosome number undergo malignant transformation (irregular shaped cells represent malignant cells; ROS, reactive oxygen species; CIN, chromosomal instability; GIN, genomic instability).
### Table 1. Deregulated expression of SAC genes across tumors

| Tumor type                      | Expression level | Main observations                                                                 | Detection method     | Reference(s) |
|---------------------------------|------------------|------------------------------------------------------------------------------------|----------------------|--------------|
| **BUB1**                        |                  |                                                                                    |                      |              |
| Acute myeloid leukemia          | Up               | Associated with −5/del(5q) therapy-related AML (CD34⁺ stem/progenitor cells)      | GEM                  | 100          |
|                                 | Down             |                                                                                    | RT-PCR               | 101          |
| Breast cancer                   | Up               | Associated with cases diagnosed at <40 years of age, estrogen- and progesterone-receptor negative tumors, high grade, and poor OS and RFS | qPCR, GEM, IHC       | 102–104      |
|                                 | Up               | Compared to normal tissue samples                                                 | GEM, IHC             | 105          |
| Clear cell renal cell carcinoma | Up               | Correlated with the number of genomic copy number changes and high Furhman grade   | qPCR                 | 106          |
|                                 | Up               | Compared to normal tissue samples                                                 | GEM                  | 105          |
| Colon cancer                    | Up               | Compared to normal tissue samples                                                 | GEM                  | 105          |
| Endometrial cancer              | +/-Up            | 28.6% of cases, associated with low clinical stage and histological grade; higher in nonendometrioid compared to endometrioid carcinomas | IHC, GEM             | 107,108      |
| Gastric cancer                  | Up               | 40–84% of cases, associated with Ki-67 expression and PCNA marker, not correlated with ploidy | qPCR, RT-PCR         | 109–111      |
|                                 | Low Freq         | Associated with larger tumor size, higher incidence of lymph node metastases, distant metastases and higher UICC stage, reduced Ki-67 protein expression and shorter survival | IHC                  | 112          |
| Glioma                          | Up               | Associated with high grade                                                        | qPCR, IHC            | 113          |
| Hepatocellular carcinoma        | Up               | Part of a gene expression signature predicting OS and DFS                          | RNAseq, GEM          | 114,115      |
|                                 | Up               | Compared to normal tissue samples                                                 | GEM                  | 105          |
| Lung cancer                     | Up               | Associated with adverse OS and RFS; progressive increase in expression from adenocarcinoma to squamous cell carcinoma, large cell carcinoma and the small cell subtype | qPCR, GEM           | 116,117      |
|                                 | Up               | Compared to normal tissue samples                                                 | GEM                  | 105          |
| Melanoma                        | Up               | Associated with metastatic melanoma                                               | GEM                  | 118          |
| Ovarian/Uterine cancer          | Up               | Compared to normal tissue samples                                                 | GEM                  | 105          |
| Pheochromocytoma and paraganglioma | Up          | Associated with metastatic tumor                                                 | RNAseq               | 119          |
| Prostate cancer                 | Up               | Compared to normal tissue samples                                                 | GEM                  | 105          |
| Salivary gland tumors           | Up               | Associated with advanced clinical stage and Ki-67 labeling index                  | qPCR and WB         | 120          |
| Thyroid Carcinomas              | Up               | Associated with undifferentiated carcinoma                                         | qPCR                 | 121          |
| **BUBR1**                       |                  |                                                                                    |                      |              |
| Acute Myeloid Leukemia          | Down             | Reduced in total bone marrow cells as well as in CD34⁺ bone marrow cells          | GEM                  | 122          |
| Bladder cancer                  | Up               | Associated with CIN, aneuploidy, centrosome amplification, high histological grade, advanced pathological stage, high cell proliferation, shorter RFS and PFS | IHC                  | 123          |
| Breast cancer                   | Up               | 38% of cases, associated with triple negative tumors, poor OS, DFS and disease-specific survival, improved OS in basal-like tumors and worse OS in luminal and untreated patients, grade 2 and 3 ductal breast cancer; correlated with high histological tumor grade, Ki-67 proliferation index and intrachromosomal instability in ductal breast cancer | IHC, GEM, IHC, qPCR | 104-126–128 |
|                                 | Up               | Compared to normal tissue samples                                                 | RNAseq, GEM          | 105          |
|                                 | Up               |                                                                                    | qPCR                 | 106          |

(Continues)
| Tumor type                                  | Expression level | Main observations                                                                                           | Detection method      | Reference(s) |
|--------------------------------------------|------------------|------------------------------------------------------------------------------------------------------------|-----------------------|--------------|
| Clear cell renal cell carcinoma            | Up               | Correlated with the number of genomic copy number changes and high Furhman grade                           | GEM                   | 105          |
| Colon cancer                               | Up               | Compared to normal tissue samples                                                                        | GEM                   | 105          |
| Colorectal cancer                          | Down             | Reduced in aneuploid compared to diploid cases                                                            | IHC                   | 129          |
| Epithelial ovarian cancer                  | +                | Associated with advanced stage, serous histology and high grade, shorter RFS                              | IHC                   | 130          |
| Esophageal squamous cell carcinoma         | Up               | Ab array, GEM, IHC, WB                                                                                     |                       | 131          |
| Gallbladder cancer                         | Up               | qPCR                                                                                                       |                       | 132          |
| Gastric cancer                             | Up               | 50–68% of cases, correlated with Ki-67 expression, aneuploidy (debated), deep invasion, lymph node and liver metastasis, poor prognosis | IHC, qPCR             | 109,133,134  |
| Glioma                                     | Up               | Associated with high grade and predictor of poor prognosis                                                | GEM and qPCR          | 113          |
| Hepatocellular carcinoma                   | Up               | 45–64% of cases; associated with larger tumor size, high histological grade, advanced pathological stage, reduced OS and RFS; associated with p53 and Ki-67 markers | qPCR, WB, IHC, RNAseq, GEM | 114,135,136  |
| Lung cancer                                | Up               | Compared to normal tissue samples                                                                        | GEM                   | 105          |
| Malignant peripheral nerve sheath tumors   | Up               | Associated with malignant transformation of plexiform neurofibroma                                         | GEM, IHC              | 137          |
| Multiple myeloma                           | Up               | Associated with high risk                                                                                  | GEM                   | 138          |
| Nasopharyngeal carcinoma                   | Up               | Commonly upregulated across six different studies                                                          | GEM                   | 139          |
| Oral squamous cell carcinoma               | Up               | Controversial results across studies: upregulated in 22.4% of cases, associated with advanced stages, larger tumor size, shorter OS and HPV-positivity (76% of cases) according to Lira et al.; associated with less advanced pathologic stage, longer OS and shorter RFS according to Rizzardi et al. | IHC, qPCR             | 140,141      |
| Ovarian cancer                             | Up               | Associated with serous carcinomas, advanced stage, and increased cellular proliferation                   | IHC                   | 142          |
| Ovarian/Uterine cancer                     | Up               | Compared to normal tissue samples                                                                        | GEM                   | 105          |
| Pancreatic cancer                          | Low              | 65% of cases                                                                                               | IHC                   | 143          |
| Pediatric adrenocortical tumors            | Up               | Associated with Weiss score 3                                                                            | qPCR                  | 145          |
| Pheochromocytoma and paraganglioma         | Up               | Associated with metastatic tumor                                                                           | RNAseq                | 119          |
| Primary gastrointestinal diffuse B cell lymphoma | Up                | Correlates with Ki-67 proliferation index, not with survival                                               | IHC                   | 146          |
| Prostate cancer                            | Up               | 63% of cases, associated with reduced OS, high Gleason score, and predictor of shorter RFS                 | IHC, qPCR             | 147,148      |
| Salivary duct carcinoma                    | Up               | Compared to normal tissue samples                                                                        | GEM                   | 105          |
| Testicular germ cell tumor                 | Down             | Decreased in nonseminomas compared to seminomas                                                            | IHC                   | 129          |
| Tumor type                        | Expression level | Main observations                                                                 | Detection method | Reference(s) |
|----------------------------------|------------------|-----------------------------------------------------------------------------------|------------------|--------------|
| Thyroid Carcinomas               | Up               | Associated with undifferentiated carcinoma, followed by advanced differentiated carcinoma | qPCR             | 121          |
| Tonsillar carcinomas             | +                | 16% of positive cells (median number); prognostic factor in univariate survival analysis and in multivariate analyses (together with stage, age, and HPV status) | IHC              | 150          |
| Upper tract urothelial carcinoma | Up               | Associated with CIN, high histological grade, shorter disease-specific survival     | IHC              | 151          |
| Wilms Tumors                     | Down             | Associated with hyperdiploid or near-or-pseudodiploid tumor, while expression levels are increased in diploid tumors | WB               | 152          |
| **CDC20**                        |                  |                                                                                    |                  |              |
| Breast cancer                    | Up               | Associated with aneuploidy, aggressive course, and poor OS                         | qPCR, IHC        | 104,153      |
|                                 | Up               | Compared to normal tissue samples                                                 | RNAseq, GEM      | 105          |
| Cervical cancer                  | Up               | 8% of low-grade squamous intraepithelial lesions, 49.6% of high-grade squamous intraepithelial lesions, 22.3% of squamous cell carcinomas | GEM, IHC         | 154,155      |
| Clear cell renal cell carcinoma  | Up               | Associated with advanced pathologic stage and shorter OS                           | GEM              | 156          |
| Colon cancer                     | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Colorectal cancer                | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Gastrointestinal cancer          | Up               | Associated with III and IV clinical stage, N classification, M0 classification, moderate pathologic differentiation, shorter OS; increased in liver metastasis | GEM, qPCR, IHC   | 157,158      |
| Gastric cancer                   | Up               | Associated with increased tumor size, histological grade, lymph node involvement, TNM stage and poor OS; independent predictor of OS | qPCR, IHC        | 159          |
| Glioblastoma                     | Up               | 74.1% of cases                                                                    | GEM, IHC         | 160          |
| Glioma                           | Up               | Associated with high grade                                                        | GEM, qPCR        | 113          |
| Head and neck tumors             | Up               | Hub gene, associated with poor tumor differentiation, high TNM stage, P53 and Ki-67 expression | GEM, qPCR        | 161-163      |
| Hepatocellular carcinoma         | Up               | Hub gene, associated with poor tumor differentiation, high TNM stage, P53 and Ki-67 expression | qPCR, WB, IHC, GEM | 115,136,161-163 |
|                                 | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Kidney renal clear cell carcinoma| Up               | Associated with poor OS                                                           | RNAseq           | 164          |
| Lung cancer                      | Up               | Hub gene, associated with poor tumor differentiation, high TNM stage, P53 and Ki-67 expression, shorter OS | IHC, RNAseq, GEM | 117,165-167 |
|                                 | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Multiple myeloma                 | Up               | Associated with high-risk patients and poor prognosis                              | GEM              | 168          |
| Myelodysplastic syndrome         | Up               | Increased in patients with dysmegakaryopoiesis, thrombocytopenia and high-risk cases, associated with increased bone marrow cellularity, age, severe thrombocytopenia, three-lineage dysplasia, complex karyotype, and worse prognosis | qPCR, IHC        | 169-171      |
| Oral squamous cell carcinoma     | Up               | 56.9% of cases, associated with shorter OS                                         | IHC              | 172          |
| Ovarian/Uterine cancer           | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Pancreatic cancer                | Up               | Associated with poor differentiation, reduced RFS in pancreatic ductal adenocarcinoma | WB, GEM, IHC     | 143,173,174 |
| Prostate cancer                  | Up               | Associated with lower biochemical-RFS after laparoscopic radical prostatectomy     | IHC              | 175          |
|                                 | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |

(Continues)
Table 1. Continued

| Tumor type                          | Expression level | Main observations                                                                 | Detection method | Reference(s) |
|-------------------------------------|------------------|------------------------------------------------------------------------------------|------------------|--------------|
| Serous epithelial ovarian cancer    | Up               | Associated with poor OS                                                            | IHC              | 176          |
| Urothelial bladder cancer           | Up               | 59% of cases, associated with high grade, advanced age and stage, nonpapillary growth pattern and distant metastasis; predictor of poor OS and RFS | IHC              | 177          |
| Uterine leiomyosarcoma              | Up               |                                                                                    | GEM              | 178          |
| MAD1                                |                  |                                                                                    |                  |              |
| Breast cancer                       | Up               | 60% of cases; associated with lymph node involvement, tumor size, grade, TP53 mutations, poor OS; not associated with increased proliferation rate and estrogen receptor status | IHC, qPCR, GEM   | 104,179      |
| Chromophobe renal cell carcinoma    | Up               | Compared to normal tissue samples                                                 | RNAseq, GEM      | 105          |
| Clear cell renal cell carcinoma     | Down             |                                                                                    | qPCR             | 180          |
| Colon cancer                        | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Gastric carcinoma                   | Down             | 47.1% of adenomas and 60.5% carcinomas, associated with advanced carcinomas and intestinal type | 2-DE, pPCR, IHC, WB | 181,182     |
| Glioma                              | Up               | Associated with high grade                                                         | GEM and qPCR     | 113          |
| Hepatocellular carcinoma            | Down             | Associated with tumor recurrence after surgical resection                          | WB               | 183          |
| Lung cancer                         | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Ovarian/Uterine cancer              | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Small cell lung cancer              | +                | 39.8% of primary tumors and 46.9% of lymph node metastasis; associated with high tumor-node-metastasis stage and International Association for the Study of Lung Cancer stage, increased tumor size and recurrence, shorter OS and RFS | IHC              | 184          |
| MAD2                                |                  |                                                                                    |                  |              |
| Breast cancer                       | Up               | 28.4% of cases; associated with age <50 years, HER-2 and PS53 positivity, luminal B and HER-2 subtypes, estrogen and progesterone-receptor negative tumors, high grade and poor OS and RFS; overexpressed in invasive ductal breast carcinoma | IHC, GEM, qPCR   | 103,104,128,185 |
|                                    | Down             | Associated with HER-2 overexpression in ductal breast carcinoma                    | IHC              | 125          |
| Cervical cancer                     | Up               | Compared to normal tissue samples                                                 | RNAseq, GEM      | 105          |
| Clear cell renal cell carcinoma     | Up               |                                                                                    | qPCR             | 106          |
| Colon cancer                        | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Colorectal cancer                   | Up               | 75% of cases, associated with increased stage, poor differentiation, presence of lymph node metastasis, and reduced survival after excision | IHC, GEM, qPCR   | 157,186      |

(Continues)
Table 1. Continued

| Tumor type                                      | Expression level | Main observations                                                                                         | Detection method                  | Reference(s) |
|------------------------------------------------|------------------|-----------------------------------------------------------------------------------------------------------|-----------------------------------|---------------|
| Endometrial cancer                              | +                | 85.7% of cases, associated with high clinical stage and histological grade                                 | IHC                               | 107           |
| Esophageal squamous cell carcinoma              | Up               | Associated with low histological grade                                                                     | AbM, GEM, IHC, WB                 | 131           |
| Gastric cancer                                  | Up               | Associated with poor differentiation, and presence of lymph node metastasis                                | IHC                               | 187           |
| Glioma                                          | Up               | Associated with high grade                                                                                  | GEM and qPCR                      | 113           |
| Hepatocellular carcinoma                       | Up               | Associated with histologic grade progression and low OS                                                   | GEM, qPCR, IHC, WB, GEM           | 115,136,188   |
| High-grade serous epithelial ovarian cancer     | Down             | Associated with reduced PFS                                                                                | IHC                               | 189           |
| Lung cancer                                     | Up               | 26.3% of cases, associated with male sex, tumor progression, visceral or parietal pleural invasion, nonadenocarcinoma, histological classification, smoking history and shorter OS and RFS; independent prognostic factor in multivariate analysis; associated with CDC20 expression in nonsmall cell lung cancer | IHC, GEM                          | 117,165,190,191 |
| Malignant pleural mesothelioma                  | Up               | Compared to normal tissue samples                                                                          | GEM                               | 105           |
| Multiple myeloma                                | Down             | Hub gene                                                                                                   | GEM                               | 192           |
| Myelodysplastic syndrome                        | Down             | Decreased in patients with 2 or 3 cytopenias and hypoplastic cases, associated with high frequency of chromosomal alterations and high mortality rate | qPCR                              | 169           |
| Nasopharyngeal carcinoma                        | Up               | Commonly upregulated across six different studies                                                          | GEM                               | 139           |
| Oral squamous cell carcinoma                    | Up               | 36.7% of cases; associated with advanced stages, larger tumor size, poor differentiation histological grade, lymph nodes involvement, high Ki-67 labeling index, shorter OS | IHC, qPCR                         | 141,194       |
| Osteosarcoma                                    | Up               | Associated with low differentiation and high clinical stage, earlier metastasis and poor OS              | IHC                               | 195           |
| Ovarian cancer                                  | Down             | Associated with increased cellular proliferation, shorter OS, and RFS                                      | IHC                               | 142,196       |
| Papillary renal cell carcinoma                  | Up               | Compared to normal tissue samples                                                                          | GEM                               | 105           |
| Primary gastrointestinal diffuse large B cell lymphoma | Up             | Associated with Ki-67 proliferation index and lower DFS                                                  | IHC                               | 199           |
| Prostate cancer                                 | Up               | Compared to normal tissue samples                                                                          | GEM                               | 105           |
| Salivary duct carcinoma                         | Up               | 55.6% of cases, no prognostic value                                                                       | IHC                               | 149           |
| Soft-tissue sarcoma                             | Up               | 52% of translocation-associated (TA, atypical or high-grade morphology, such as round cell liposarcoma and fibrosarcomatous dermatofibrosarcoma protuberans) and 66% of non-TA sarcoma; associated with multipolar mitoses and anaphase bridges | IHC                               | 200           |
various oncogenic backgrounds. Recently, Benezra’s group showed that individual chromosome loss in tetraploid MEFs may drive tumorigenesis by favoring anchorage-independent growth, DNA damage, and CIN.56 These results are highly relevant within the context of tumors with increased ploidy and may suggest a difference between the tumorigenic potential of single-chromosome gain under normal ploidy. In line with previous studies reporting a negative effect of chromosome number alterations on cellular fitness, aneuploidy is one of the hallmark[s of cancer, a disease of cells undergoing uncontrolled proliferation. According to the Mitelman Database, about 90% of solid tumors and 50% of hematological neoplasms are aneuploid.57 How can this be reconciled with the findings described so far in this section?

Despite the detrimental effect of chromosome number alterations on cellular fitness, aneuploidy is one of the hallmarks of cancer, a disease of cells undergoing uncontrolled proliferation. According to the Mitelman Database, about 90% of solid tumors and 50% of hematological neoplasms are aneuploid.57 How can this be reconciled with the findings described so far in this section?

Although in vitro culturing of fibroblasts from patients with constitutional aneuploidy have shown that whole chromosome gains do not confer levels of CIN comparable to those observed in cancer cells,53 several studies indicate a correlation or a causal relationship between aneuploidy and genome/CIN. A correlation between the two phenomena has been observed in monosomic and trisomic models. Amniocytes from trisomic fetuses display a higher incidence of random aneuploidy,58 and lymphocytes from patients with Turner’s syndrome or constitutional autosomal trisomy (of chromosome 21, 18, or 13) are prone to develop nonchromosome specific aneuploidy under phytohemagglutinin stimulation.59,60 Moreover, genomic instability (GIN) is proportional to the degree of aneuploidy in transformed Chinese hamster embryo cells.61 GIN and CIN are key features of the adaptive response driven by aneuploidy and favoring malignant transformation (Fig. 3b). Single-chromosome aneuploidy is sufficient to induce CIN, an increase in double strand break (DSB) during DNA replication and defective DSB repair, resulting in a “mutator phenotype” in yeasts.62 Indeed, yeast strains with high proliferative capacity display aneuploidy-tolerating mutations,63 including both strain-specific genetic lesions and common mutations shared between different aneuploid strains. Common lesions mainly target the ubiquitin-proteasome machinery, with recurrent loss-of-function mutations in the gene encoding the deubiquitinating enzyme UBP6. Therefore, aneuploidy is maintained and propagated through the positive selection of cells that evolve and become fitter. Increased DNA damage, genomic rearrangements and replication stress have also been reported in human

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**Table 1. Continued**

| Tumor type                  | Expression level | Main observations                                                                 | Detection method | Reference(s) |
|-----------------------------|------------------|------------------------------------------------------------------------------------|------------------|--------------|
| Testicular germ cell tumor  | Down             | Decreased nuclear expression and increased cytoplasmic levels in seminomas, decreased in nonseminomas compared to seminomas | IHC              | 129,201      |
| Thyroid carcinomas          | Up               | Associated with undifferentiated carcinoma, followed by advanced differentiated carcinoma | qPCR             | 121          |
| Tonsillar carcinomas        | +                | 27% of positive cells (median number)                                             | IHC              | 150          |
| Urothelial bladder cancer   | Up               | 51% of cases, associated with high grade, advanced stage and nonpapillary growth pattern, predictor of poor OS | IHC              | 177          |
| BUB3                        |                  |                                                                                    |                  |              |
| Breast cancer               | Up               | Significantly overexpressed when amplified in triple negative breast cancer        | qPCR, GEM       | 104,202      |
| Clear cell renal cell       | Up               | Compared to normal tissue samples                                                 | RNAseq, GEM     | 105          |
| carcinoma                   |                  |                                                                                    |                  |              |
| Colon cancer                | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Gastric cancer              | Up               | 79% of cases, correlates with Ki-67 expression, does not correlate with ploidy      | qPCR             | 109          |
| Hepatocellular carcinoma    | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Lung cancer                 | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Ovarian/Uterine cancer      | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |
| Prostate cancer             | Up               | Compared to normal tissue samples                                                 | GEM              | 105          |

Up, overexpressed; down, downregulated; +, positive; low freq, low frequency; low, low expression; del, deletion; AML, acute myeloid leukemia; OS, overall survival; DFS, disease-free survival; PFS, progression-free survival; PCNA, proliferating cell nuclear antigen; UICC, International Union Against Cancer; HPV, human papillomavirus; pT, pathological tumor progression; TNM, tumor/node/metastasis; IHC, immunohistochemistry; GEM, gene expression microarray; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; WB, western blotting; RNAseq, RNA sequencing; 2-DE, two-dimensional gel electrophoresis.
cells as a consequence of aneuploidy. In particular, chromosome segregation errors (specifically chromatin bridges, which typically arise as a result of DNA damage) can lead to the accumulation of postmitotic DNA damage due to the “trapping” of chromatin bridges in the cleavage furrow. Moreover, trisomic colon cancer cells display a higher rate of chromosome missegregation than that of euploid ones, and the capacity of accurate chromosome segregation decreases in a discontinuous way compared to chromosome number changes. Indeed, yeast cells with a ploidy between 1.5 and 2 are more susceptible to chromosome missegregation than those with a near haploid karyotype. This evidence suggests that additional numerical and structural chromosomal aberrations exacerbate genomic complexity in aneuploid cells. It was recently suggested that replicative stress caused by aneuploidy and oncogenic alterations targeting TP53, RB, or KRAS induce CIN, even in

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**Table 2. Mouse models with SAC gene overexpression or downregulation showing evidence of increased/reduced predisposition to tumor development**

| Mouse model     | Phenotype                                                                 | Reference |
|-----------------|---------------------------------------------------------------------------|-----------|
| **MAD2-tg**     | • Aneuploid and tetraploid cells, chromosomal breaks and fragments, end-to-end fusions (dicentric and acentric chromosomes), chromatid breaks and gaps<br>• 50% of mice were dead by 75 weeks<br>• Prone to develop hepatoma and hepatocellular carcinoma, lung adenomas, fibrosarcomas and lymphomas | 31        |
| **mad2+/−**     | • Defective mitotic checkpoint and chromosome missegregation<br>• High rate of papillary lung adenocarcinomas in aged mice | 32        |
| **mad1+/−**     | • Aneuploidy<br>• Prone to develop lung adenocarcinoma, hepatocellular carcinoma, rhabdomyosarcoma, osteosarcoma, hemangiosarcoma and uterine sarcoma (twofold increase) by 18 months of age | 203       |
| **BUB1-tg**     | • Chromosome missegregation due to misalignment and near diploid aneuploidy<br>• Prone to develop d lymphomas, lipomas, sarcomas, liver and skin tumors (≥67%)<br>• Premature onset of Eµ-Myc-mediated lymphoma | 33        |
| **bubR1+/−**    | • Defective in SAC activation, reduced securin and CDC20 expression, increased level of micronuclei<br>• No effects on the frequency or rate of spontaneous tumors<br>• High incidence and premature onset of colon adenocarcinoma when primed with azoxymethane<br>• Develop lung and liver tumors when primed with azoxymethane | 204       |
| **bubR1K243R/+**| • Acetylation-defective bubR1 allele<br>• Aneuploidy, weakened SAC, premature sister chromatid separation, chromosome missegregation, increased level of micronuclei<br>• Prone to develop solid (10.7%) and hematological (12.4%) malignancies including hepatocellular carcinoma, sarcoma, adenocarcinoma, megakaryocytic leukemia, B cell lymphoma | 205       |
| **bub1Δ2−3/Δ2−3**| • Deletion of exons 2 and 3, which originates a null allele<br>• Aneuploidy, defective SAC, chromosome segregation errors<br>• 76% of (129/B6) bub1Δ2−3/Δ2−3 mice, 42% of bub1Δ2−3/+ mice and 28% of bub1+/+ mice developed tumors by 23 to 25 months of age (liver, lung and brain tumors) | 34        |
| **BUBR1-tg**    | • Genomic integrity is preserved through correction of mitotic checkpoint impairment and microtubule-kinetochore attachment defects<br>• Resistance to RAS-mediated tumorigenesis | 14        |
| **bub1−/H bub1H/H bub1*/−** | • Weakened mitotic checkpoint and aneuploidy, with a milder phenotype and a higher BUB1 expression in bub1+/− mice<br>• bub1−/H: prone to develop sarcomas, lymphomas, and lung tumor<br>• bub1H/H: prone to develop sarcomas and highly susceptible to hepatocellular carcinomas<br>• bub1*/−: decreased tumor incidence, especially in the liver and the lung | 35        |
| **bub3*/−**     | • Aneuploidy, premature sister-chromatid separation and chromatid breaks<br>• No effects on the frequency or rate of spontaneous tumors | 206       |
| **rae1−/− bub3*/−** | • Defective mitotic checkpoint and chromosome missegregation<br>• Prone to develop carcinogen-induced lung tumors | 207       |
| **cdc20+/AAA cdc20+/−, cdc20H/H, cdc20−/H** | • Mutation to alanine of three residues in the MAD2-binding site.<br>• Functional loss of SAC, premature anaphase and aneuploidy<br>• Prone to develop tumors (90% by 24 months of age), especially hepatomas and lymphomas | 208       |
| **cdc20+/−, cdc20H/H, cdc20−/−** | • Chromosome misalignment, chromatin bridging, delayed anaphase onset<br>• Progressive aneuploidy according to CDC20 expression level<br>• No effects on the frequency or rate of spontaneous tumors<br>• No effects on the frequency of carcinogen-induced lung tumors | 209       |

Tg, transgenic; H, hypomorphic allele.
the absence of mutations in genes involved in chromosome segregation or mitotic checkpoint. For example, in immortalized colonic epithelial cells that acquire an extra copy of chromosome 7 under the selective pressure of serum-free culture conditions, the expression of oncogenic KRAS or the depletion of TP53 predispose to the acquisition of trisomy 20. Thus, the progressive accumulation of mutations, translocations and/or copy number variants improves cell tolerability toward the negative consequences of the altered chromosome number and promotes cell growth, as demonstrated in yeasts, trisomic MEFs and human cell lines. Moreover, aneuploidy confers an evolutionary flexibility that may contribute, along with oncogenic events, to the cellular heterogeneity observed in cancer and to the aggressive phenotype of advanced malignancies with complex karyotypes.

Aneuploidy itself is an evolutionary strategy to compensate for the deletion of evolvable genes in yeasts. Although, these genes regulate essential cellular processes (e.g., Golgi vesicle trafficking, nuclear transport and nuclear pore complex, protein targeting to endoplasmic reticulum), the cells can survive their loss by developing alternative strategies, including altered gene dosage induced by aneuploidy. For example, aneuploidization can correct failure of cytokinesis in MYO1-deficient yeasts. Altered levels of transcription factors encoded by aneuploid chromosomes induce changes in the expression of downstream genes involved in cytokinesis. Different patterns of aneuploidy, arising in the absence of MYO1, converge to common targets capable of restoring cytokinesis.

Specific biological and metabolic properties contribute to the adaptive response induced by aneuploidy (Fig. 3b). Aneuploid cells show heightened anchorage-independent growth and migration capacity (e.g., in a colorectal model). In human pluripotent stem cells (hPSCs), aneuploidy inhibits differentiation propensity and apoptosis, increases proliferation and favors the formation of teratomas characterized by a gene expression profile resembling that of germ cell tumors. Aneuploid cells can also redistribute their resources to overcome functional defects. For example, yeast cells use aneuploidy to survive telomerase insufficiency by increasing the expression of the telomerase components at the expense of ribosome synthesis. The deregulated expression of genes that are involved in oxidative phosphorylation and that protect from oxidative stress also contributes to the tumor-promoting effect of aneuploidy. Yeasts can adapt to deficiency of all thiol peroxidases, the enzymes that alleviate oxidative stress-mediated DNA damage, by acquiring an extra copy of chromosome XI. This allows the removal of hydrogen peroxide through increased expression of the mitochondrial proteins CCP1 and UTH1 and enforced respiration. Therefore, the evolutionary flexibility induced by aneuploidy is not only an attempt to survive a disadvantageous chromosome number, but also a favorable condition in some settings. A recent study suggested a role of aneuploidy in immune escape (Fig. 3b). Most aneuploid tumors show decreased neoantigen load, possibly mediated by limited neoantigen generation and presentation through the MHC complex. This, in turn, results in decreased immune cell infiltration and makes aneuploid neo-plasms less responsive to immunomodulating agents.

Overall, evidence obtained in nonmalignant cells and cancer models indicates that aneuploidy, which is detrimental per se, can be beneficial and even favor the development and selection of aggressive malignant clones by enabling cells to modulate independent pathways simultaneously and to explore a wide phenotypic landscape.

Aneuploidy and Cancer: Cell Type, Genomic Background and Environmental Conditions Matter
Although Down’s syndrome patients have a 10-fold lower solid tumor-related mortality than the general population, they are more likely to develop leukemia. A gain of chromosome 21 is a common event in sporadic leukemia, is the most frequent karyotypic alteration in acute lymphoblastic leukemia, but is rare in glioblastoma, breast, and colorectal cancer. These observations are suggestive of a tissue- and chromosome-specific oncogenic effect of aneuploidy. Accordingly, malignant transformation does not occur randomly in the majority of transgenic and knock-out mouse models of aneuploidy (Table 2). MAD2 overexpression specifically increases the susceptibility to hepatoma and hepatocellular carcinoma, lung adenoma, fibrosarcoma, and lymphoma. Reduced BUB1 expression favors the development of thymic lymphoma and colon cancer in p53+/− and apoCMin/+ mice, respectively, but suppresses prostatic intraepithelial neoplasia in pten+/− mice and does not alter the frequency of pituitary tumors and sarcoma formation in the rb+/− model. Moreover, cultured hPSCs tend to acquire trisomy of chromosome 12, which is the most common chromosomal aberration in germ cell tumors, and trisomy of chromosome 13, which confers a distinctive cytokinesis failure phenotype to colon cancer cells.

Although the spectrum and degree of aneuploidy differ among tumors, many human cancers share recurrent aneuploidies. It is currently believed that tumor-specific aneuploidies coexist with recurrent aneuploidies across tumors. Using a computational approach, Davoli et al. showed that chromosome number alterations do not occur randomly; a selective pressure forces the acquisition of oncogenes and the loss of tumor suppressors, as observed in yeast cells that overcome MYO1 deficiency by preferential gain of specific chromosomes.

Aneuploidy also improves cellular adaptation to specific biological and environmental features. In nonpathological conditions aneuploidy is well tolerated under “population flush” effects when rapid cell expansion is needed (e.g., during embryogenesis) and in nonregenerating tissues, as brain and liver, in which the nonproliferative cellular status is protective against the potentially dangerous consequences of aneuploidy. On the contrary, aneuploidy is physiologically selected against in tissues that undergo self-renewal, including the hematopoietic compartment, skin, and intestines.
However, aneuploidy improves the survival rate under conditions of stress, including extreme temperature or pH, lack of nutrients, and incubation with chemotherapeutic or antifungal agents in budding yeasts.44 Similarly, trisomic colorectal cancer cell lines have a proliferative advantage over euploid cells under hypoxic conditions, chemotherapeutic pressure and in conditions of serum starvation.23 Serum-free conditions also favor the acquisition of trisomy 7 in immortalized human colonic epithelial cells.70 The pathogen Candida Albicans develops aneuploidy, mainly consisting in chromosome 5 trisomy (or gain of an isochromosome composed of the two left arms of chromosome 5), as an adaptive strategy to resist fluconazole, commonly used to treat fungal infections.82 The aneuploid strain has an increased growth rate compared to the wild-type strain under fluconazole pressure, but this advantage is lost in the absence of the drug. Moreover, the accumulation of multiple trisomies of chromosome 3–7 during fluconazole treatment increases resistance, while also having a low fitness cost under nonselective conditions in this model.83 Similarly, Chen et al. showed that dynamic karyotype changes allow yeast cells to survive drug exposure.84

This complex scenario recapitulates tumor biology given that both cancer and leukemia stem cells localize mainly in hypoxic niches. If we consider aneuploid cells as a premalignant state, their genomic plasticity confers the ability to evolve to a malignant phenotype in order to tolerate adverse environmental conditions. The DNA replication stress, which fuels defective chromosome condensation and segregation in aneuploid hPSCs,69 may also propagate GIN in cancer stem cells. Karyotypic heterogeneity may in turn result in phenotypic variations, thus allowing specific aneuploid cells to be fitter under conditions of stress, including oncogene withdrawal and pharmacological treatment. CIN induced by MAD2 overexpression sustains tumor progression and recurrence upon oncogene inactivation in KrasG12D models of breast85 and lung68 cancer, respectively, through activation of alternative oncogenic signaling pathways. In human and murine medulloblastoma, GIN and aneuploidy are common features of the malignant clone driving relapse, which originates from a minor clone present at diagnosis and selected by therapy.86 Similarly, CIN and aneuploidy characterize chemotherapy-resistant subclones, giving rise to metastases in breast cancer.87 This suggests that aneuploidy, when causing moderate levels of genetic instability, can improve adaptation to the microenvironmental conditions in a specific tumor site, without compromising cell viability.

**Therapeutic Potential of Aneuploidy in Cancer Patients**

Recent studies have shown that exacerbating chromosome missegregation rates in aneuploid cells by combining heterozygous deletions of the centromere-linked motor protein cenp-E and of the SAC component mad2, results in high CIN levels and leads to tumor suppression in mice due to the induction of cell death.88 High CIN levels sustain tumor-initiating cells while depleting mature tumor cells.89 For example, CENP-E reduction in apoptosisMin/+ mice does not inhibit the formation of intestinal tumors but hampers their progression.89 This suggests a dual relationship between aneuploid malignancies and antitumor therapeutic strategies: aneuploidy can be a cancer strength or an Achilles’ heel.

According to two different clinical trials on metastatic melanoma, aneuploidy promotes cancer immune escape and correlates with bad prognosis in response to immune checkpoint blockade agents.75 However, chromosomally unstable tumors, such as those with aneuploidy, may be induced to mitotic catastrophe by drugs interfering with chromosome segregation, in particular by enhancing the chromosome missegregation rate.90,91 These include compounds that disrupt microtubule dynamics either by inducing overpolymerization (stabilizing drugs, e.g., taxanes) or by reducing polymerization (destabilizing drugs, e.g., vinblastine), or drugs that disrupt the kinetochore-microtubule attachment, correction of misattachments (e.g., Aurora B inhibitors) or SAC activity. For example, the microtubule-stabilizing drug paclitaxel kills breast cancer cells by inducing chromosome missegregation on multipolar spindles.92 These preclinical tests need to be substantiated by clinical studies. Recently, a clinical trial to compare paclitaxel response with CIN level in breast cancer patients began its recruitment phase (NCT03096418, https://clinicaltrials.gov). The downside of mitotic drugs is their severe bone marrow toxicity. However, this can be prevented by ad hoc combination therapies, including a chemotherapy backbone, aimed at tumor debulking, disease eradication, and a reduction in side effects. The controversial role of the mitotic checkpoint in the response to antimitotic drugs93 should also be taken into account when designing clinical trials. A successful approach can be built on the concept of synthetic lethality, which refers to the simultaneous perturbation of two genes resulting in the death of the cell or the organism. Certain drugs can cause lethality in malignant cells carrying structural or functional alterations in specific genes or pathways. For example, cancer cell lines with defective chromatin cohesion are resistant to paclitaxel but highly responsive to SAC inhibition,94 and knock-down of Ppp2r1a is lethal in MAD2 overexpressing tumors.95 These “lethal” combinations should be exploited to target aneuploidy-supporting cellular functions. Indeed, in addition to their neutropenic effects, mitotic drugs are not expected to be effective against tumors showing a negative correlation between CIN and survival. The strength of these aneuploid tumors resides in their increased tolerability toward stress conditions, their genomic complexity and stem cell-like quiescence, which probably favor resistance to chemotherapy and maintenance of proliferative capacity,96 driving progression to a very aggressive phenotype.

The aneuploid cell-dependence on chaperone pathways and heightened protein turnover suggest additional therapeutic potential, exploiting proteotoxic stress as aneuploidy-related vulnerability. Aneuploid MEFs, hPSCs, human embryonic stem...
cells, colorectal cancer cells lines and induced (i)PSCs from trisomy 21 fibroblasts are sensitive to compounds, inhibiting protein folding (17-(Allylaminio)-17-demethoxygeldanamycin), or inducing energy stress (aminomidazole carboxamide ribonucleotide) although a comparison between aneuploid and euploid cells, led to different results among the models. Moreover, hPSCs with trisomy 12 showed enhanced sensitivity to drugs targeting DNA replication, including etoposide, cytarabine and gemcitabine hydrochloride, compared to euploid cells. Taken together, these findings indicate that the aneuploid condition offers a therapeutic window for specific antitumor treatment strategies.

Conclusions
An improved understanding of the molecular mechanisms of aneuploidy and of its consequences on cell physiology has provided important insights into the complex relationship between chromosome number alterations and cancer. Aneuploidy can increase malignant cell strength while also creating vulnerability to specific conditions or therapeutic interventions. The tissue type, genetic background and microenvironment play a pivotal role in the match. However, the genetic determinants of the protumorigenic or antitumorigenic effects of aneuploidy and their interplay with the biology of the cell of origin remain unclear. Altered expression of SAC components is a common feature across cancer types and minority of cases carry mutated SAC genes, which may interfere with chromosome segregation fidelity. However, chromosome segregation represents a rapid event in the eukaryotic cell cycle. Cells exiting the quiescent G0 phase accumulate mass, activate signaling pathways, replicate the genome and prepare for mitotic division through G1, S and G2 phases. Dysfunction of cellular components involved in these stages through either mutations, copy number alterations, epigenetic modifications, or deregulated expression may compromise mitotic fidelity and favor aneuploidy. Thus, the identification of genomic patterns that associate and/or synergize with aneuploid phenotypic profiles in promoting tumor development might be a prerequisite to any therapeutic decision, along with the definition of chromosome missegregation frequencies inducing adaptive levels of CIN. These approaches will unravel the relationship between genetic variability, drug resistance and acquisition of stem cell characteristics, while also defining lineage-specific vulnerabilities for aneuploid tumors. Such knowledge, complemented by the availability of rationally designed targeted agents that have produced promising results, will serve as a map for personalized synthetic lethal therapeutic strategies against aneuploid tumors.

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