IJC
International Journal of Cancer

Aneuploidy: Cancer strength or vulnerability?

Giorgia Simonetti[1], Samantha Bruno[1], Antonella Padella[1], Elena Tenti[1] and Giovanni Martinelli[2]

[1]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna and Institute of Hematology "L. e A. Seràgnoli", Bologna, Italy
[2]Scientific Directorate, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy

Mini Review

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 aneuploidization 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

Key words: aneuploidy, carcinogenesis, cancer therapy, spindle assembly checkpoint

Abbreviations: APC: APC, WNT signaling pathway regulator; APC/C: anaphase-promoting complex/cyclosome; APP: Amyloid Beta Precursor Protein; BUB1: BUB1 Mitotic Checkpoint Serine/Threonine Kinase; BUB3: BUB3, Mitotic Checkpoint Protein; BUBR1/BUB1B: BUB1 Mitotic Checkpoint Serine/Threonine Kinase B; CCP1: cytochrome-c peroxidase; CDC20: Cell Division Cycle 20; CDK1: Cyclin Dependent Kinase 1; CENP-E: centromere protein E; CIN: chromosome instability; DSB: double strand break; FISH: Fluorescence In Situ Hybridization; GIN: genomic instability; hPSCs: human pluripotent stem cells; KNL1: Kinetochore Scaffold 1; KRAS: KRAS proto-oncogene; MAD1/MAD1L1: MAD1 Mitotic Arrest Deficient Like 1; MAD2/MAD2L1: Mitotic Arrest Deficient 2 Like 1; MCC: mitotic checkpoint complex; MEFs: murine embryonic fibroblasts; MHC: major histocompatibility complex; MPS1: TTK Protein Kinase; MVA: Mosaic Variegated Aneuploidy; MYO1: myosin 1; NDC80: NDC80, Kinetochore Complex Component; PLK1: Polo Like Kinase 1; PPP2R1A: protein phosphatase 2 scaffold subunit alpha; PTEN: phosphatase and tensin homolog; RB: RB Transcriptional Corepressor 1; ROS: Reactive oxygen species; SAC: spindle assembly checkpoint; SKY: Spectral Karyotyping; tg: trasgenic; TP53: Tumor Protein P53; UBP6: ubiquitin-specific protease UBP6; UTH1: SUN family protein UTH1.

Conflict of interest: Dr. Martinelli reports personal fees from Amgen, personal fees from Ariad/Incyte, personal fees from Pfizer, personal fees and nonfinancial support from Roche, personal fees from Celgene, personal fees from Janssen, personal fees from Jazz Pharmaceuticals, personal fees from ABBVIE, personal fees from Novartis, nonfinancial support from Daiichi Sankyo, nonfinancial support from Shire, outside the submitted work. The other authors have nothing to disclose.

Grant sponsor: Associazione Italiana per la Ricerca sul Cancro; **Grant number:** 19226; **Grant sponsor:** European Union Seventh Framework Programme; **Grant numbers:** 306242-NGS-PTL, FP7/2007–2013

DOI: 10.1002/ijc.31718

History: Received 20 Feb 2018; Accepted 14 Jun 2018; Online 7 Jul 2018

Correspondence to: Giorgia Simonetti, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna and Institute of Hematology "L. e A. Seràgnoli," via Massarenti, 9, 40138 Bologna, Italy, E-mail: giorgia.simonetti3@unibo.it; Tel: 0039 0512143791

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.[1] They can revert to diploidy and become aneuploid,[2] while diploid liver cells can increase their ploidy. This dynamic mechanism, defined as "ploidy-conveyor,"[3] generates aneuploidy and has also been reported in human hepatocytes.[4] Under conditions of stress, hepatocytes acquire specific aneuploidies enabling them to resist to chronic liver injuries.[5] Moreover, aneuploid neurons, retaining functional activity,[6] have been observed in developing and adult murine models[7,8] and in the human brain.[9,10] The majority of studies have reported a prevalence of aneuploid cells exceeding 50% and 20% in the liver[3,4] and brain,[7,10–12] 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[13] 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[14–16] and cerebral degeneration,[17] with deficits in neural progenitor proliferation and maturation.[18] In oocytes, the frequency of chromosome segregation errors in meiosis I increase with maternal aging,[19] along with a decrease in BUBR1 protein.[20] 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,[21] and buccal cells from patients with Alzheimer's disease frequently carry trisomy of chromosomes 21 or 17, where many susceptibility genes are located.[22]

These findings suggest that a low frequency of aneuploid cells can be tolerated[13] or may even be advantageous under specific conditions in nonmalignant tissues,[23] whereas increased rates of aneuploidy can become pathogenic, as observed in neurodegenerative diseases[22] and in cancer.[24] Theodor Boveri initially suggested that an abnormal chromosome number causes tumorigenesis.[24] 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.[25,26] 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 $(APC/C)^{CDC20}$.[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/, Fig. 2). 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[28] and chromosome instability (CIN), which have also been detected in a *p53* mutant mouse model.[29] 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

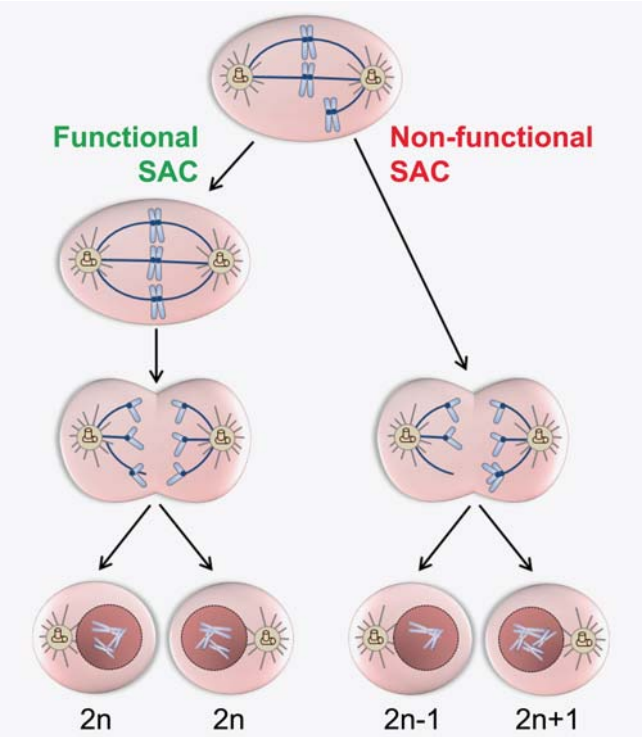

Figure 1. Generation of aneuploidy by non-functional SAC. The SAC is activated by the presence of unattached or misaligned kinetochores and prevents chromosome segregation errors. A non-functional SAC allows cells with unattached or misaligned kinetochores to proceed from metaphase to anaphase, resulting in daughter cells with an abnormal chromosome number.

overexpressed or downregulated. For example, CDC20 overexpression impairs SAC function and favors aneuploidization in oral cancer.[30] Moreover, chromosome missegregation and aneuploidy have been reported in both transgenic (tg), hypomorphic and haploinsufficient mouse models, including MAD2-tg[31] and *mad2*$^{+/-}$,[32] *BUB1*-tg,[33] *bub1*$^{\Delta 2-3/\Delta 2-3}$,[34] *bub1*$^{-/H}$ and *bub1*$^{H/H}$.[35] 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*$^{H/H}$ and *bub1*$^{-/H}$ mice and lower tumor incidence.[35] However, SAC gene deficiency is also detrimental, probably due to extreme CIN.[26] 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[28,31]), and to potential oncogenic roles of SAC proteins outside mitosis: BUBR1 plays a role in DNA damage responses,[36] CDC20 is involved in the regulation of apoptosis,[37] DNA repair[38] and stem-like cell properties[39–41] and MAD1 has a function at the Golgi apparatus.[42] 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.[43–48] Changes in chromosome copy number result in transcriptomic alterations and gene-dosage effects at the proteomic level, as extensively reviewed by Ried *et al.*[49] 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).[50,51] 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. 3*a*). First, aneuploidy is an extremely rare event in normal conditions, even in brain and liver.[13] Second, trisomic murine embryonic fibroblasts (MEFs) show contact inhibition properties, proliferation arrest in low-

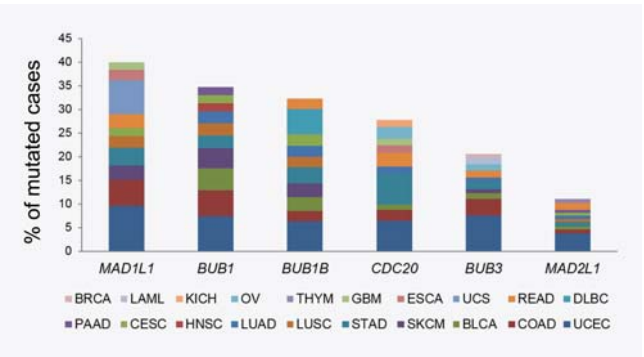

Figure 2. Distribution of SAC gene mutations across cancers. Frequency of patients with mutations in SAC genes from TCGA cohorts (LAML, Acute Myeloid Leukemia; BLCA, Bladder Urothelial Carcinoma; BRCA, Breast Invasive Carcinoma; CESC, Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma; COAD, Colon Adenocarcinoma; ESCA, Esophageal Carcinoma; GBM, Glioblastoma Multiforme; HNSC, Head and Neck Squamous Cell Carcinoma; KICH, Kidney Chromophobe; LUAD, Lung Adenocarcinoma; LUSC, Lung Squamous Cell Carcinoma; DLBC, Diffuse Large B-cell Lymphoma; OV, Ovarian Serous Cystadenocarcinoma; PAAD, Pancreatic Adenocarcinoma; READ, Rectum Adenocarcinoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach Adenocarcinoma; THYM, Thymoma; UCS, Uterine Carcinosarcoma; UCEC, Uterine Corpus Endometrial Carcinoma).

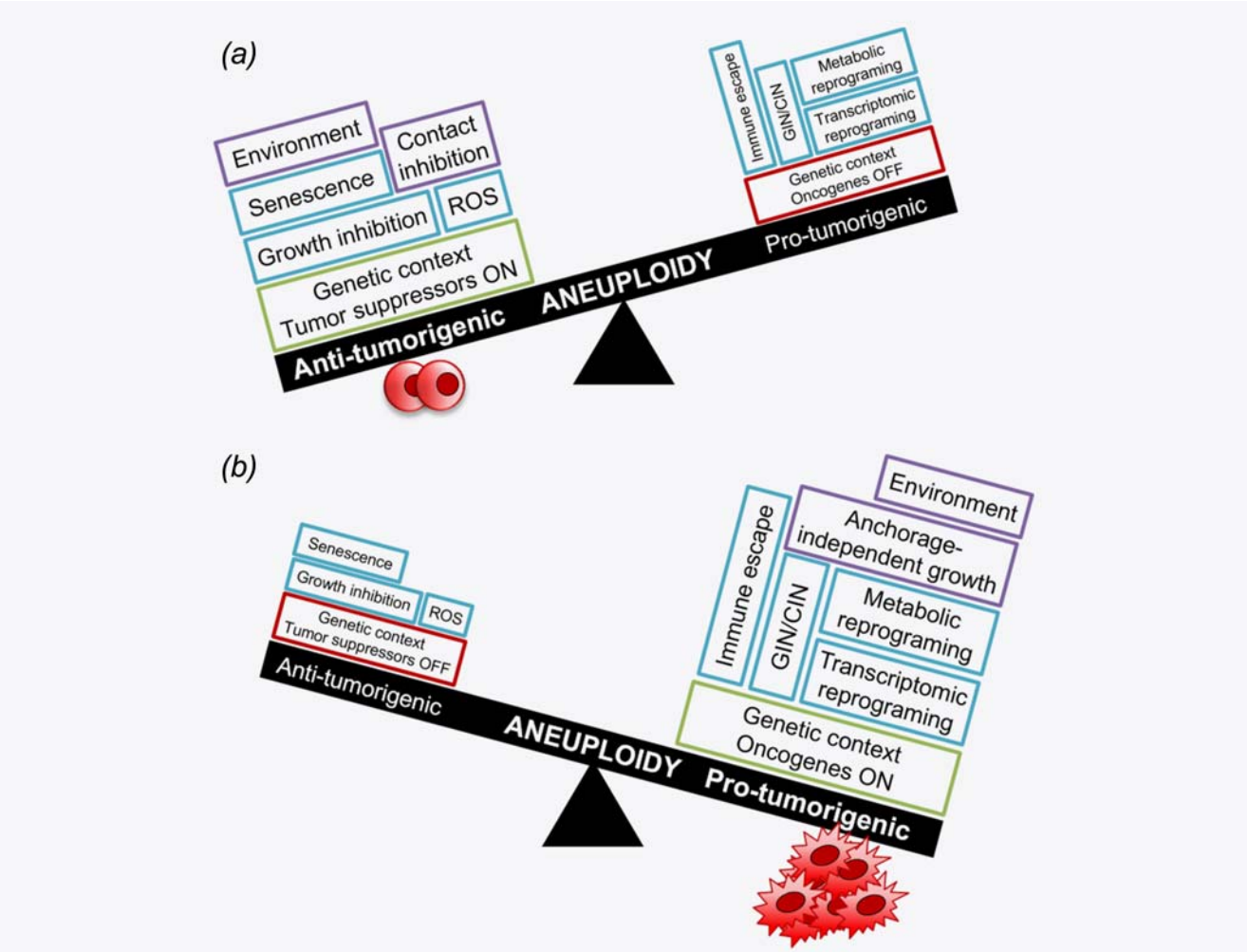

Figure 3. The complex relationship between aneuploidy and cancer. (*a*) Aneuploidy-related growth and contact inhibition, ROS production, cell senescence can cooperate with environmental conditions and tumor suppressor activity to inhibit malignant transformation (round shaped cells represent nontransformed 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).

serum medium, lack of clonogenic capacity and senescence features after 7–10 passages in culture.[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.[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.[53] Moreover, constitutional aneuploidy *per se* is not sufficient to generate tumor-like CIN.[53] Aneuploid cells modulate their metabolic and transcriptional programs to improve their fitness. Indeed, aneuploidy is associated with higher glucose and/or glutamine consumption[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.[44,45,54] These pathways are deregulated in several aneuploid cell lines, although the specific combinations of genes displaying altered expression differ.[51]

These observations, mostly obtained from trisomic models, indicate that single-chromosome aneuploidy is not sufficient *per se* to induce malignant transformation[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.[55] Moreover, some mouse models with deregulated SAC genes that develop aneuploidy have a decreased rate of tumorigenesis (Table 2), even under

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	RNAseq, GEM	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	GEM, qPCR	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	104,124–128
	Up	Compared to normal tissue samples	RNAseq, GEM	105
	Up		qPCR	106

(Continues)

Table 1. Continued

Tumor type	Expression level	Main observations	Detection method	Reference(s)
Clear cell renal cell carcinoma		Correlated with the number of genomic copy number changes and high Furhman grade		
	Up	Compared to normal tissue samples	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
	Up	Compared to normal tissue samples	GEM	105
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	Up		GEM	143
	Low	65% of cases	IHC	144
Pediatric adrenocortical tumors	Up	Associated with Weiss score 3	qPCR	145
Pheochromocytoma and paraganglioma	Up	Associated with metastatic tumor	RNAseq	119
Primary gastrointestinal diffuse large 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
	Up	Compared to normal tissue samples	GEM	105
Salivary duct carcinoma	Up	25.9% of cases, no prognostic value	IHC	149
Testicular germ cell tumor	Down	Decreased in nonseminomas compared to seminomas	IHC	129

(Continues)

Table 1. Continued

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, 49.4% 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
	Up	Compared to normal tissue samples	GEM	105
Colon cancer	Up	Compared to normal tissue samples	GEM	105
Colorectal 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		WB	30
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	19.6% of cases, correlated with male sex, pT status, pleural invasion, nonadenocarcinoma histology, MAD2 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
Myelodisplastc 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 epiphelial 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]
	Up	Compared to normal tissue samples	RNAseq, GEM	[105]
Chromophobe renal cell carcinoma	Down		qPCR	[180]
Clear cell renal cell carcinoma	Down		qPCR	[106]
	Up	Compared to normal tissue samples	GEM	[105]
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]
	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]
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 P53 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]
	Up	Compared to normal tissue samples	RNAseq, GEM	[105]
Cervical cancer	Up	2% of low-grade squamous intraepithelial lesions, 67.1% of high-grade squamous intraepithelial lesions, 52.4% of squamous cell carcinomas; correlated with patient age ‹60 years, non-keratinizing histologic type and a lesser degree of stromal invasion in squamous cell carcinoma cases	IHC	[154]
Clear cell renal cell carcinoma	Up		qPCR	[106]
	Up	Compared to normal tissue samples	GEM	[105]
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	qPCR, IHC, WB, GEM	[115,136,188]
	Up	Compared to normal tissue samples	GEM	[105]
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]
	Up	Compared to normal tissue samples	GEM	[105]
Malignant pleural mesothelioma	Up	Potentially correlated with reduced OS	GEM, qPCR, WB, IHC	[192]
Multiple myeloma	Down	Hub gene	GEM	[193]
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]
	Up	Associated with increased bone marrow cellularity and age, severe thrombocytopenia, poor prognosis	IHC, qPCR	[170,171]
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]
	Up	52.3% of cases of high-grade serous carcinoma, where low expression predicts inferior PFS; overexpressed in malignant mucinous ovarian cancer compared to non-malignant and benign lesions	IHC	[197,198]
Ovarian/Uterine cancer	Up	Compared to normal tissue samples	GEM	[105]
Papillary renal cell carcinoma	Up		qPCR	[180]
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]

(Continues)

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
	Up	Compared to normal tissue samples	RNAseq, GEM	105
Clear cell renal cell carcinoma	Up	Compared to normal tissue samples	GEM	105
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; RFS, recurrence-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.

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 chromosome gain and loss. However, single-chromosome loss did not induce transformation of diploid MEFs, indicating a ploidy-specific effect. Moreover, single-chromosome loss hampered the proliferative capacity of diploid hematopoietic cells, in line with previous studies reporting a negative effect of single-chromosome gain under normal ploidy.

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. 3*b*). 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

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 • 50% of mice were dead by 75 weeks • Prone to develop hepatoma and hepatocellular carcinoma, lung adenomas, fibrosarcomas and lymphomas	31
$mad2^{+/-}$	• Defective mitotic checkpoint and chromosome missegregation • High rate of papillary lung adenocarcinomas in aged mice	32
$mad1^{+/-}$	• Aneuploidy • 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 • Prone to develop d lymphomas, lipomas, sarcomas, liver and skin tumors (≈67%) • Premature onset of *Eμ-Myc*-mediated lymphoma	33
$bubR1^{+/-}$	• Defective in SAC activation, reduced securin and CDC20 expression, increased level of micronuclei • No effects on the frequency or rate of spontaneous tumors • High incidence and premature onset of colon adenocarcinoma when primed with azoxymethane • Develop lung and liver tumors when primed with azoxymethane	204
$bubR1^{K243R/+}$	• Acetylation-defective *bubR1* allele • Aneuploidy, weakened SAC, premature sister chromatid separation, chromosome missegregation, increased level of micronuclei • Prone to develop solid (10.7%) and hematological (12.4%) malignancies including hepatocellular carcinoma, sarcoma, adenocarcinoma, megakaryocytic leukemia, B cell lymphoma	205
$bub1^{\Delta 2-3/\Delta 2-3}$	• Deletion of exons 2 and 3, which originates a null allele • Aneuploidy, defective SAC, chromosome segregation errors • 76% of (129/B6) $bub1^{\Delta 2-3/\Delta 2-3}$ mice, 42% of $bub1^{\Delta 2-3/+}$ 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 • Resistance to RAS-mediated tumorigenesis	14
$bub1^{-/H}$ $bub1^{H/H}$ $bub1^{+/-}$	• Weakened mitotic checkpoint and aneuploidy, with a milder phenotype and a higher BUB1 expression in $bub1^{+/-}$ mice • $bub1^{-/H}$: prone to develop sarcomas, lymphomas, and lung tumor • $bub1^{H/H}$: prone to develop sarcomas and highly susceptible to hepatocellular carcinomas • $bub1^{+/-}$: decreased tumor incidence, especially in the liver and the lung	35
$bub3^{+/-}$	• Aneuploidy, premature sister-chromatid separation and chromatid breaks • No effects on the frequency or rate of spontaneous tumors	206
$rae1^{+/-}$ $bub3^{+/-}$	• Defective mitotic checkpoint and chromosome missegregation • Prone to develop carcinogen-induced lung tumors	207
$cdc20^{+/AAA}$	• Mutation to alanine of three residues in the MAD2-binding site. • Functional loss of SAC, premature anaphase and aneuploidy • Prone to develop tumors (50% by 24 months of age), especially hepatomas and lymphomas	208
$cdc20^{+/-}$, $cdc20^{H/H}$, $cdc20^{-/H}$	• Chromosome misalignment, chromatin bridging, delayed anaphase onset • Progressive aneuploidy according to CDC20 expression level • No effects on the frequency or rate of spontaneous tumors • No effects on the frequency of carcinogen-induced lung tumors	209

Tg, transgenic; H, hypomorphic allele.

cells as a consequence of aneuploidy.[64] 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.[65] Moreover, trisomic colon cancer cells display a higher rate of chromosome missegregation than that of euploid ones,[66] and the capacity of accurate chromosome segregation decreases in a discontinuous way compared to chromosome number changes.[67] Indeed, yeast cells with a ploidy between 1.5 and 2 are more susceptible to chromosome missegregation than those with a near haploid karyotype.[67] 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[64] and oncogenic alterations targeting *TP53*, *RB*,[29] or *KRAS*[68] induce CIN,[69] even in

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.[70] 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[52] and human cell lines.[64] 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 vescicle 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.[71] For example, aneuploidization can correct failure of cytokinesis in MYO1-deficient yeasts.[72] 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. 3*b*). Aneuploid cells show heightened anchorage-independent growth and migration capacity (e.g., in a colorectal model[23]). 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.[43] Aneuploid cells can also redistribute their resources to overcome functional defects.[73] For example, yeast cells use aneuploidy to survive telomerase insufficiency by increasing the expression of the telomerase components at the expense of ribosome synthesis.[73] 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.[74] 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[75] (Fig. 3*b*). 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 neoplasms 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.[55] 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.[76] 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.[31] Reduced BUB1 expression favors the development of thymic lymphoma and colon cancer in $tp53^{+/-}$ and $apc^{Min/+}$ 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.[77] Moreover, cultured hPSCs tend to acquire trisomy of chromosome 12, which is the most common chromosomal aberration in germ cell tumors,[43] and trisomy of chromosome 13, which confers a distinctive cytokinesis failure phenotype to colon cancer cells.[66]

Although the spectrum and degree of aneuploidy differ among tumors, many human cancers share recurrent aneuploidies.[78] 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,[79] as observed in yeast cells that overcome MYO1 deficiency by preferential gain of specific chromosomes.[72]

Aneuploidy also improves cellular adaptation to specific biological and environmental features. In nonpathological conditions aneuploidy is well tolerated under "population flush" effects,[80] 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.[81]

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 $Kras^{G12D}$ models of breast[85] and lung[68] 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 $apc^{Min/+}$ 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 drugs[93] 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 chromatid 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 cell lines and induced (i)PSCs from trisomy 21 fibroblasts are sensitive to compounds, inhibiting protein folding (17-(Allylamino)-17-demethoxygeldanamycin), or inducing energy stress (aminoimidazole carboxamide ribonucleotide) although a comparison between aneuploid and euploid cells, led to different results among the models.[43,97–99] Moreover, hPSCs with trisomy 12 showed enhanced sensitivity to drugs targeting DNA replication, including etoposide, cytarabine and gemcitabine hydrochloride, compared to euploid cells.[43] 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.

References

1. Gupta S. Hepatic polyploidy and liver growth control. *Semin Cancer Biol* 2000;10:161–71.
2. Faggioli F, Vezzoni P, Montagna C. Single-cell analysis of ploidy and centrosomes underscores the peculiarity of normal hepatocytes. *PloS One* 2011;6:e26080.
3. Duncan AW, Taylor MH, Hickey RD, et al. The ploidy conveyor of mature hepatocytes as a source of genetic variation. *Nature* 2010;467: 707–10.
4. Duncan AW, Hanlon Newell AE, Smith L, et al. Frequent aneuploidy among normal human hepatocytes. *Gastroenterology* 2012; 142:25–8.
5. Duncan AW, Hanlon Newell AE, Bi W, et al. Aneuploidy as a mechanism for stress-induced liver adaptation. *J Clin Invest* 2012;122:3307–15.
6. Kingsbury MA, Friedman B, McConnell MJ, et al. Aneuploid neurons are functionally active and integrated into brain circuitry. *Proc Natl Acad Sci USA* 2005;102:6143–7.
7. Rehen SK, McConnell MJ, Kaushal D, et al. Chromosomal variation in neurons of the developing and adult mammalian nervous system. *Proc Natl Acad Sci USA* 2001;98:13361–6.
8. Yang AH, Kaushal D, Rehen SK, et al. Chromosome segregation defects contribute to aneuploidy in normal neural progenitor cells. *J Neurosci* 2003;23:10454–62.
9. Yurov YB, Iourov IY, Monakhov VV, et al. The variation of aneuploidy frequency in the developing and adult human brain revealed by an interphase FISH study. *J Histochem Cytochem* 2005;53:385–90.
10. Yurov YB, Iourov IY, Vorsanova SG, et al. Aneuploidy and confined chromosomal mosaicism in the developing human brain. *PloS One* 2007;2: e558.
11. Pack SD, Weil RJ, Vortmeyer AO, et al. Individual adult human neurons display aneuploidy: detection by fluorescence in situ hybridization and single neuron PCR. *Cell Cycle* 2005;4: 1758–60.
12. Rehen SK, Yung YC, McCreight MP, et al. Constitutional aneuploidy in the normal human brain. *J Neurosci* 2005;25:2176–80.
13. Knouse KA, Wu J, Whittaker CA, et al. Single cell sequencing reveals low levels of aneuploidy across mammalian tissues. *Proc Natl Acad Sci USA* 2014;111:13409–4.
14. Baker DJ, Dawlaty MM, Wijshake T, et al. Increased expression of BubR1 protects against aneuploidy and cancer and extends healthy lifespan. *Nat Cell Biol* 2013;15:96–102.
15. Wijshake T, Malureanu LA, Baker DJ, et al. Reduced life- and healthspan in mice carrying a mono-allelic BubR1 MVA mutation. *PLoS Genet* 2012;8:e1003138.
16. Baker DJ, Jeganathan KB, Cameron JD, et al. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat Genet* 2004;36:744–9.
17. Hartman TK, Wengenack TM, Poduslo JF, et al. Mutant mice with small amounts of BubR1 display accelerated age-related gliosis. *Neurobiol Aging* 2007;28:921–7.
18. Yang Z, Jun H, Choi CI, et al. Age-related decline in BubR1 impairs adult hippocampal neurogenesis. *Aging Cell* 2017;16:598–601.
19. Nagaoka SI, Hassold TJ, Hunt PA. Human aneuploidy: mechanisms and new insights into an age-old problem. *Nat Rev Genet* 2012;13:493–504.
20. Riris S, Webster P, Homer H. Digital multiplexed mRNA analysis of functionally important genes in single human oocytes and correlation of changes in transcript levels with oocyte protein expression. *Fertil Steril* 2014;101: 857–64.
21. Wisniewski KE, Wisniewski HM, Wen GY. Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. *Ann Neurol* 1985;17:278–82.
22. Thomas P, Fenech M. Chromosome 17 and 21 aneuploidy in buccal cells is increased with ageing and in Alzheimer's disease. *Mutagenesis* 2008;23:57–65.
23. Rutledge SD, Douglas TA, Nicholson JM, et al. Selective advantage of trisomic human cells cultured in non-standard conditions. *Sci Rep* 2016; 6:22828.
24. Boveri T, Boveri M. *The origin of malignant tumorsed*. Baltimore: The Williams & Wilkins Company, 1929. ix, 119
25. Nicholson JM, Cimini D. How mitotic errors contribute to karyotypic diversity in cancer. *Adv Cancer Res* 2011;112:43–75.
26. Kops GJ, Weaver BA, Cleveland DW. On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat Rev Cancer* 2005;5:773–85.
27. Musacchio A. The molecular biology of spindle assembly checkpoint signaling dynamics. *Curr Biol* 2015;25:R1002–18.
28. Hernando E, Nahle Z, Juan G, et al. Rb inactivation promotes genomic instability by uncoupling cell cycle progression from mitotic control. *Nature* 2004;430:797–802.
29. Schvartzman JM, Duijf PH, Sotillo R, et al. Mad2 is a critical mediator of the chromosome

instability observed upon Rb and p53 pathway inhibition. *Cancer Cell* 2011;19:701–14.
30. Mondal G, Sengupta S, Panda CK, et al. Overexpression of Cdc20 leads to impairment of the spindle assembly checkpoint and aneuploidization in oral cancer. *Carcinogenesis* 2007;28: 81–92.
31. Sotillo R, Hernando E, Diaz-Rodriguez E, et al. Mad2 overexpression promotes aneuploidy and tumorigenesis in mice. *Cancer Cell* 2007;11:9–23.
32. Michel LS, Liberal V, Chatterjee A, et al. MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. *Nature* 2001;409:355–9.
33. Ricke RM, Jeganathan KB, van Deursen JM. Bub1 overexpression induces aneuploidy and tumor formation through aurora B kinase hyperactivation. *J Cell Biol* 2011;193:1049–64.
34. Schliekelman M, Cowley DO, O'Quinn R, et al. Impaired Bub1 function in vivo compromises tension-dependent checkpoint function leading to aneuploidy and tumorigenesis. *Cancer Res* 2009;69:45–54.
35. Jeganathan K, Malureanu L, Baker DJ, et al. Bub1 mediates cell death in response to chromosome missegregation and acts to suppress spontaneous tumorigenesis. *J Cell Biol* 2007;179: 255–67.
36. Fang Y, Liu T, Wang X, et al. BubR1 is involved in regulation of DNA damage responses. *Oncogene* 2006;25:3598–605.
37. Wan L, Tan M, Yang J, et al. APC(Cdc20) suppresses apoptosis through targeting Bim for ubiquitination and destruction. *Dev Cell* 2014;29: 377–91.
38. Cho HJ, Lee EH, Han SH, et al. Degradation of human RAP80 is cell cycle regulated by Cdc20 and Cdh1 ubiquitin ligases. *Mol Cancer Res* 2012;10:615–25.
39. Hadjihannas MV, Bernkopf DB, Bruckner M, et al. Cell cycle control of Wnt/beta-catenin signalling by conductin/axin2 through CDC20. *EMBO Rep* 2012;13:347–54.
40. Mao DD, Gujar AD, Mahlokozera T, et al. A CDC20-APC/SOX2 signaling Axis regulates human Glioblastoma stem-like cells. *Cell Rep* 2015;11:1809–21.
41. Xie Q, Wu Q, Mack SC, et al. CDC20 maintains tumor initiating cells. *Oncotarget* 2015;6: 13241–54.
42. Wan J, Zhu F, Zasadil LM, et al. Golgi-localized pool of the mitotic checkpoint component Mad1 controls integrin secretion and cell migration. *Curr Biol* 2014;24:2687–92.
43. Ben-David U, Arad G, Weissbein U, et al. Aneuploidy induces profound changes in gene expression, proliferation and tumorigenicity of human pluripotent stem cells. *Nat Commun* 2014;5:4825.
44. Pavelka N, Rancati G, Zhu J, et al. Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast. *Nature* 2010; 468:321–5.
45. Sheltzer JM, Torres EM, Dunham MJ, et al. Transcriptional consequences of aneuploidy. *Proc Natl Acad Sci USA* 2012;109:12644–9.
46. Torres EM, Sokolsky T, Tucker CM, et al. Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science* 2007;317: 916–24.
47. Upender MB, Habermann JK, McShane LM, et al. Chromosome transfer induced aneuploidy results in complex dysregulation of the cellular transcriptome in immortalized and cancer cells. *Cancer Res* 2004;64:6941–9.
48. Williams BR, Prabhu VR, Hunter KE, et al. Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells. *Science* 2008;322:703–9.
49. Ried T, Hu Y, Difilippantonio MJ, et al. The consequences of chromosomal aneuploidy on the transcriptome of cancer cells. *Biochim Biophys Acta* 2012;1819:784–93.
50. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. *Nat Rev Drug Discov* 2013;12:931–47.
51. Dephoure N, Hwang S, O'Sullivan C, et al. Quantitative proteomic analysis reveals post-translational responses to aneuploidy in yeast. *Elife* 2014;3:e03023.
52. Sheltzer JM, Ko JH, Replogle JM, et al. Single-chromosome gains commonly function as tumor suppressors. *Cancer Cell* 2017;31:240–55.
53. Valind A, Jin Y, Baldetorp B, et al. Whole chromosome gain does not in itself confer cancer-like chromosomal instability. *Proc Natl Acad Sci USA* 2013;110:21119–3.
54. Durrbaum M, Kuznetsova AY, Passerini V, et al. Unique features of the transcriptional response to model aneuploidy in human cells. *BMC Genomics* 2014;15:139.
55. Yang Q, Rasmussen SA, Friedman JM. Mortality associated with Down's syndrome in the USA from 1983 to 1997: a population-based study. *Lancet* 2002;359:1019–25.
56. Thomas R, Marks DH, Chin Y, et al. Whole chromosome loss and associated breakage-fusion-bridge cycles transform mouse tetraploid cells. *EMBO J* 2018;37:201–18.
57. Mitelman F, Johansson B, Mertens F. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, http://cgap.nci.nih.gov/Chromosomes/Mitelman 2017.
58. Biron-Shental T, Liberman M, Sharvit M, et al. Amniocytes from aneuploidy embryos have enhanced random aneuploidy and signs of senescence - can these findings be related to medical problems? *Gene* 2015;562:232–5.
59. Reish O, Regev M, Kanesky A, et al. Sporadic aneuploidy in PHA-stimulated lymphocytes of trisomies 21, 18, and 13. *Cytogenet Genome Res* 2011;133:184–9.
60. Reish O, Brosh N, Gobazov R, et al. Sporadic aneuploidy in PHA-stimulated lymphocytes of Turner's syndrome patients. *Chromosome Res* 2006;14:527–34.
61. Duesberg P, Rausch C, Rasnick D, et al. Genetic instability of cancer cells is proportional to their degree of aneuploidy. *Proc Natl Acad Sci USA* 1998;95:13692–7.
62. Sheltzer JM, Blank HM, Pfau SJ, et al. Aneuploidy drives genomic instability in yeast. *Science* 2011;333:1026–30.
63. Torres EM, Dephoure N, Panneerselvam A, et al. Identification of aneuploidy-tolerating mutations. *Cell* 2010;143:71–83.
64. Passerini V, Ozeri-Galai E, de Pagter MS, et al. The presence of extra chromosomes leads to genomic instability. *Nat Commun* 2016;7:10754.
65. Janssen A, van der Burg M, Szuhai K, et al. Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. *Science* 2011;333:1895–8.
66. Nicholson JM, Macedo JC, Mattingly AJ, et al. Chromosome mis-segregation and cytokinesis failure in trisomic human cells. *Elife* 2015;4: e05068.
67. Zhu J, Pavelka N, Bradford WD, et al. Karyotypic determinants of chromosome instability in aneuploid budding yeast. *PLoS Genet* 2012;8: e1002719.
68. Sotillo R, Schvartzman JM, Socci ND, et al. Mad2-induced chromosome instability leads to lung tumour relapse after oncogene withdrawal. *Nature* 2010;464:436–0.
69. Lamm N, Ben-David U, Golan-Lev T, et al. Genomic instability in human pluripotent stem cells arises from replicative stress and chromosome condensation defects. *Cell Stem Cell* 2016; 18:253–61.
70. Ly P, Eskiocak U, Kim SB, et al. Characterization of aneuploid populations with trisomy 7 and 20 derived from diploid human colonic epithelial cells. *Neoplasia* 2011;13:348–57.
71. Liu G, Yong MY, Yurieva M, et al. Gene essentiality is a quantitative property linked to cellular Evolvability. *Cell* 2015;163:1388–99.
72. Rancati G, Pavelka N, Fleharty B, et al. Aneuploidy underlies rapid adaptive evolution of yeast cells deprived of a conserved cytokinesis motor. *Cell* 2008;135:879–93.
73. Millet C, Ausiannikava D, Le Bihan T, et al. Cell populations can use aneuploidy to survive telomerase insufficiency. *Nat Commun* 2015;6: 8664.
74. Kaya A, Gerashchenko MV, Seim I, et al. Adaptive aneuploidy protects against thiol peroxidase deficiency by increasing respiration via key mitochondrial proteins. *Proc Natl Acad Sci USA* 2015;112:10685–90.
75. Davoli T, Uno H, Wooten EC, et al. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. *Science* 2017;355:eaaf8399.
76. Nicholson JM, Cimini D. Cancer karyotypes: survival of the fittest. *Front Oncol* 2013;3:148.
77. Baker DJ, Jin F, Jeganathan KB, et al. Whole chromosome instability caused by Bub1 insufficiency drives tumorigenesis through tumor suppressor gene loss of heterozygosity. *Cancer Cell* 2009;16:475–86.
78. Zack TI, Schumacher SE, Carter SL, et al. Pan-cancer patterns of somatic copy number alteration. *Nat Genet* 2013;45:1134–40.
79. Davoli T, Xu AW, Mengwasser KE, et al. Cumulative haploinsufficiency and triplosensitivity drive aneuploidy patterns and shape the cancer genome. *Cell* 2013;155:948–62.
80. Carson H. The population flush and its genetic consequences. In: Lewontin R, ed *Population biology and evolution*. Syracuse, NY: Syracuse University Press, 1968. 123–37.
81. Pfau SJ, Silberman RE, Knouse KA, et al. Aneuploidy impairs hematopoietic stem cell fitness and is selected against in regenerating tissues in vivo. *Genes Dev* 2016;30:1395–408.
82. Selmecki A, Forche A, Berman J. Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. *Science* 2006;313: 367–70.
83. Selmecki AM, Dulmage K, Cowen LE, et al. Acquisition of aneuploidy provides increased fitness during the evolution of antifungal drug resistance. *PLoS Genet* 2009;5:e1000705.

84. Chen G, Mulla WA, Kucharavy A, et al. Targeting the adaptability of heterogeneous aneuploids. *Cell* 2015;160:771–84.
85. Rowald K, Mantovan M, Passos J, et al. Negative selection and chromosome instability induced by Mad2 overexpression delay breast Cancer but facilitate oncogene-independent outgrowth. *Cell Rep* 2016;15:2679–91.
86. Morrissy AS, Garzia L, Shih DJ, et al. Divergent clonal selection dominates medulloblastoma at recurrence. *Nature* 2016;529:351–7.
87. Yates LR, Gerstung M, Knappskog S, et al. Subclonal diversification of primary breast cancer revealed by multiregion sequencing. *Nat Med* 2015;21:751–9.
88. Silk AD, Zasadil LM, Holland AJ, et al. Chromosome missegregation rate predicts whether aneuploidy will promote or suppress tumors. *Proc Natl Acad Sci USA* 2013;110:E4134–41.
89. Zasadil LM, Britigan EM, Ryan SD, et al. High rates of chromosome missegregation suppress tumor progression but do not inhibit tumor initiation. *Mol Biol Cell* 2016;27:1981–9.
90. Kops GJ, Foltz DR, Cleveland DW. Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. *Proc Natl Acad Sci USA* 2004;101:8699–704.
91. Janssen A, Kops GJ, Medema RH. Elevating the frequency of chromosome mis-segregation as a strategy to kill tumor cells. *Proc Natl Acad Sci USA* 2009;106:19108–3.
92. Zasadil LM, Andersen KA, Yeum D, et al. Cytotoxicity of paclitaxel in breast cancer is due to chromosome missegregation on multipolar spindles. *Sci Transl Med* 2014;6:229ra43.
93. Yamada HY, Gorbsky GJ. Spindle checkpoint function and cellular sensitivity to antimitotic drugs. *Mol Cancer Ther* 2006;5:2963–9.
94. de Lange J, Faramarz A, Oostra AB, et al. Defective sister chromatid cohesion is synthetically lethal with impaired APC/C function. *Nat Commun* 2015;6:8399.
95. Bian Y, Kitagawa R, Bansal PK, et al. Synthetic genetic array screen identifies PP2A as a therapeutic target in Mad2-overexpressing tumors. *Proc Natl Acad Sci USA* 2014;111:1628–33.
96. Kusumbe AP, Bapat SA. Cancer stem cells and aneuploid populations within developing tumors are the major determinants of tumor dormancy. *Cancer Res* 2009;69:9245–53.
97. Tang YC, Williams BR, Siegel JJ, et al. Identification of aneuploidy-selective antiproliferation compounds. *Cell* 2011;144:499–512.
98. Li LB, Chang KH, Wang PR, et al. Trisomy correction in down syndrome induced pluripotent stem cells. *Cell Stem Cell* 2012;11:615–9.
99. Ly P, Kim SB, Kaisani AA, et al. Aneuploid human colonic epithelial cells are sensitive to AICAR-induced growth inhibition through EGFR degradation. *Oncogene* 2013;32:3139–46.
100. Qian Z, Fernald AA, Godley LA, et al. Expression profiling of CD34+ hematopoietic stem/progenitor cells reveals distinct subtypes of therapy-related acute myeloid leukemia. *Proc Natl Acad Sci USA* 2002;99:14925–30.
101. Lin SF, Lin PM, Yang MC, et al. Expression of hBUB1 in acute myeloid leukemia. *Leuk Lymphoma* 2002;43:385–91.
102. Johnson RH, Hu P, Fan C, et al. Gene expression in "young adult type" breast cancer: a retrospective analysis. *Oncotarget* 2015;6:13688–702.
103. Wang Z, Katsaros D, Shen Y, et al. Biological and clinical significance of MAD2L1 and BUB1, genes frequently appearing in expression signatures for breast Cancer prognosis. *PloS One* 2015;10:e0136246.
104. Yuan B, Xu Y, Woo JH, et al. Increased expression of mitotic checkpoint genes in breast cancer cells with chromosomal instability. *Clin Cancer Res* 2006;12:405–10.
105. Thiru P, Kern DM, McKinley KL, et al. Kinetochore genes are coordinately up-regulated in human tumors as part of a FoxM1-related cell division program. *Mol Biol Cell* 2014;25:1983–94.
106. Pinto M, Vieira J, Ribeiro FR, et al. Overexpression of the mitotic checkpoint genes BUB1 and BUBR1 is associated with genomic complexity in clear cell kidney carcinomas. *Cellular Oncol* 2008;30:389–95.
107. Li L, Xu DB, Zhao XL, et al. Combination analysis of Bub1 and Mad2 expression in endometrial cancer: act as a prognostic factor in endometrial cancer. *Arch Gynecol Obstet* 2013;288:155–65.
108. Moreno-Bueno G, Sanchez-Estevez C, Cassia R, et al. Differential gene expression profile in endometrioid and nonendometrioid endometrial carcinoma: STK15 is frequently overexpressed and amplified in nonendometrioid carcinomas. *Cancer Res* 2003;63:5697–702.
109. Grabsch H, Takeno S, Parsons WJ, et al. Overexpression of the mitotic checkpoint genes BUB1, BUBR1, and BUB3 in gastric cancer--association with tumour cell proliferation. *J Pathol* 2003;200:16–22.
110. Shigeishi H, Oue N, Kuniyasu H, et al. Expression of Bub1 gene correlates with tumor proliferating activity in human gastric carcinomas. *Pathobiology* 2001;69:24–9.
111. Grabsch HI, Askham JM, Morrison EE, et al. Expression of BUB1 protein in gastric cancer correlates with the histological subtype, but not with DNA ploidy or microsatellite instability. *J Pathol* 2004;202:208–14.
112. Stahl D, Braun M, Gentles AJ, et al. Low BUB1 expression is an adverse prognostic marker in gastric adenocarcinoma. *Oncotarget* 2017;8:76329–39.
113. Bie L, Zhao G, Cheng P, et al. The accuracy of survival time prediction for patients with glioma is improved by measuring mitotic spindle checkpoint gene expression. *PloS One* 2011;6:e25631.
114. Agarwal R, Narayan J, Bhattacharyya A, et al. Gene expression profiling, pathway analysis and subtype classification reveal molecular heterogeneity in hepatocellular carcinoma and suggest subtype specific therapeutic targets. *Cancer Genet* 2017;216-217:37–51.
115. Yan H, Li Z, Shen Q, et al. Aberrant expression of cell cycle and material metabolism related genes contributes to hepatocellular carcinoma occurrence. *Pathol Res Pract* 2017;213:316–21.
116. Zhang C, Min L, Zhang L, et al. Combined analysis identifies six genes correlated with augmented malignancy from non-small cell to small cell lung cancer. *Tumour Biol* 2016;37:2193–7.
117. Jin X, Liu X, Li X, et al. Integrated analysis of DNA methylation and mRNA expression profiles data to identify key genes in lung adenocarcinoma. *Biomed Res Int* 2016;2016:4369431.
118. Riker AI, Enkemann SA, Fodstad O, et al. The gene expression profiles of primary and metastatic melanoma yields a transition point of tumor progression and metastasis. *BMC Med Genomics* 2008;1:13.
119. Suh YJ, Choe JY, Park HJ. Malignancy in Pheochromocytoma or Paraganglioma: integrative analysis of 176 cases in TCGA. *Endocr Pathol* 2017;28:159–64.
120. Shigeishi H, Yoneda S, Taki M, et al. Correlation of human Bub1 expression with tumor-proliferating activity in salivary gland tumors. *Oncol Rep* 2006;15:933–8.
121. Wada N, Yoshida A, Miyagi Y, et al. Overexpression of the mitotic spindle assembly checkpoint genes hBUB1, hBUBR1 and hMAD2 in thyroid carcinomas with aggressive nature. *Anticancer Res* 2008;28:139–44.
122. Schnerch D, Schmidts A, Follo M, et al. BubR1 is frequently repressed in acute myeloid leukemia and its re-expression sensitizes cells to antimitotic therapy. *Haematologica* 2013;98:1886–95.
123. Yamamoto Y, Matsuyama H, Chochi Y, et al. Overexpression of BUBR1 is associated with chromosomal instability in bladder cancer. *Cancer Genet Cytogenet* 2007;174:42–7.
124. Cirak Y, Furuncuoglu Y, Yapicier O, et al. Predictive and prognostic values of BubR1 and synuclein-gamma expression in breast cancer. *Int J Clin Exp Pathol* 2015;8:5345–NaN.
125. Du J, Du Q, Zhang Y, et al. Expression of cell-cycle regulatory proteins BUBR1, MAD2, aurora a, cyclin a and cyclin E in invasive ductal breast carcinomas. *Histol Histopathol* 2011;26:761–8.
126. Maciejczyk A, Szelachowska J, Czapiga B, et al. Elevated BUBR1 expression is associated with poor survival in early breast cancer patients: 15-year follow-up analysis. *J Histochem Cytochem* 2013;61:330–9.
127. Ocana A, Perez-Pena J, Diez-Gonzalez L, et al. Transcriptomic analyses identify association between mitotic kinases, PDZ-binding kinase and BUB1, and clinical outcome in breast cancer. *Breast Cancer Res Treat* 2016;156:1–8.
128. Scintu M, Vitale R, Prencipe M, et al. Genomic instability and increased expression of BUB1B and MAD2L1 genes in ductal breast carcinoma. *Cancer Lett* 2007;254:298–307.
129. Burum-Auensen E, Deangelis PM, Schjolberg AR, et al. Reduced level of the spindle checkpoint protein BUB1B is associated with aneuploidy in colorectal cancers. *Cell Prolif* 2008;41:645–59.
130. Lee YK, Choi E, Kim MA, et al. BubR1 as a prognostic marker for recurrence-free survival rates in epithelial ovarian cancers. *Br J Cancer* 2009;101:504–10.
131. Uemura N, Nakanishi Y, Kato H, et al. Antibody-based proteomics for esophageal cancer: identification of proteins in the nuclear factor-kappaB pathway and mitotic checkpoint. *Cancer Sci* 2009;100:1612–22.
132. Kim JH, Kim HN, Lee KT, et al. Gene expression profiles in gallbladder cancer: the close genetic similarity seen for early and advanced gallbladder cancers may explain the poor prognosis. *Tumour Biol* 2008;29:41–9.
133. Ando K, Kakeji Y, Kitao H, et al. High expression of BUBR1 is one of the factors for inducing DNA aneuploidy and progression in gastric cancer. *Cancer Sci* 2010;101:639–45.

134. Enjoji M, Iida S, Sugita H, et al. BubR1 and AURKB overexpression are associated with a favorable prognosis in gastric cancer. *Mol Med Rep* 2009;2:589–96.
135. Liu AW, Cai J, Zhao XL, et al. The clinicopathological significance of BUBR1 overexpression in hepatocellular carcinoma. *J Clin Pathol* 2009;62: 1003–8.
136. Jin B, Wang W, Du G, et al. Identifying hub genes and dysregulated pathways in hepatocellular carcinoma. *Eur Rev Med Pharmacol Sci* 2015; 19:592–601.
137. Stricker TP, Henriksen KJ, Tonsgard JH, et al. Expression profiling of 519 kinase genes in matched malignant peripheral nerve sheath tumor/plexiform neurofibroma samples is discriminatory and identifies mitotic regulators BUB1B, PBK and NEK2 as overexpressed with transformation. *Modern Pathol* 2013;26:930–43.
138. Yang Y, Gu C, Luo C, et al. BUB1B promotes multiple myeloma cell proliferation through CDC20/CCNB axis. *Med Oncol* 2015;32:81.
139. Huang C, Tang H, Zhang W, et al. Integrated analysis of multiple gene expression profiling datasets revealed novel gene signatures and molecular markers in nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev* 2012;21: 166–75.
140. Lira RC, Miranda FA, Guimaraes MC, et al. BUBR1 expression in benign oral lesions and squamous cell carcinomas: correlation with human papillomavirus. *Oncol Rep* 2010;23: 1027–36.
141. Rizzardi C, Torelli L, Schneider M, et al. MAD2 expression in oral squamous cell carcinoma and its relationship to tumor grade and proliferation. *Anticancer Res* 2014;34:7021–7.
142. McGrogan B, Phelan S, Fitzpatrick P, et al. Spindle assembly checkpoint protein expression correlates with cellular proliferation and shorter time to recurrence in ovarian cancer. *Hum Pathol* 2014;45:1509–9.
143. Long J, Zhang Z, Liu Z, et al. Identification of genes and pathways associated with pancreatic ductal adenocarcinoma by bioinformatics analyses. *Oncol Lett* 2016;11:1391–7.
144. Ohashi A, Ohori M, Iwai K, et al. Aneuploidy generates proteotoxic stress and DNA damage concurrently with p53-mediated post-mitotic apoptosis in SAC-impaired cells. *Nat Commun* 2015;6:7668.
145. Borges KS, Moreno DA, Martinelli CE Jr, et al. Spindle assembly checkpoint gene expression in childhood adrenocortical tumors (ACT): overexpression of aurora kinases a and B is associated with a poor prognosis. *Pediatr Blood Cancer* 2013;60:1809–6.
146. Chen F, Yang G, Xia B. Increased expression of the spindle checkpoint protein BubR1 is associated with high cell proliferation in primary gastrointestinal diffuse large B cell lymphoma. *Cell Biochem Biophys* 2013;66:747–52.
147. Cirak Y, Sarsik B, Cakar B, et al. Predictive and prognostic values of tau and BubR1 protein in prostate cancer and their relationship to the Gleason score. *Med Oncol* 2013;30:526.
148. Fu X, Chen G, Cai ZD, et al. Overexpression of BUB1B contributes to progression of prostate cancer and predicts poor outcome in patients with prostate cancer. *OncoTargets Therapy* 2016; 9:2211–0.
149. Ko YH, Roh JH, Son YI, et al. Expression of mitotic checkpoint proteins BUB1B and MAD2L1 in salivary duct carcinomas. *J Oral Pathol Med* 2010;39:349–55.
150. Hannisdal K, Burum-Auensen E, Schjolberg A, et al. Correlation between reduced expression of the spindle checkpoint protein BubR1 and bad prognosis in tonsillar carcinomas. *Head Neck* 2010;32:1354–62.
151. Yamamoto Y, Oga A, Akao J, et al. BUBR1 overexpression predicts disease-specific survival after nephroureterectomy in patients with upper tract urothelial carcinoma. *Jpn J Clin Oncol* 2016;46: 754–61.
152. Haruta M, Matsumoto Y, Izumi H, et al. Combined BubR1 protein down-regulation and RASSF1A hypermethylation in Wilms tumors with diverse cytogenetic changes. *Mol Carcinog* 2008;47:660–6.
153. Karra H, Repo H, Ahonen I, et al. Cdc20 and securin overexpression predict short-term breast cancer survival. *Br J Cancer* 2014;110:2905–13.
154. Kim Y, Choi JW, Lee JH, et al. MAD2 and CDC20 are upregulated in high-grade squamous intraepithelial lesions and squamous cell carcinomas of the uterine cervix. *Int J Gynecol Pathol* 2014;33:517–23.
155. Espinosa AM, Alfaro A, Roman-Basaure E, et al. Mitosis is a source of potential markers for screening and survival and therapeutic targets in cervical cancer. *PloS One* 2013;8:e55975.
156. Yuan L, Chen L, Qian K, et al. Co-expression network analysis identified six hub genes in association with progression and prognosis in human clear cell renal cell carcinoma (ccRCC). *Genomics Data* 2017;14:132–40.
157. Shi G, Wang Y, Zhang C, et al. Identification of genes involved in the four stages of colorectal cancer: gene expression profiling. *Mol Cell Probes* 2018;37:39–47.
158. Wu WJ, Hu KS, Wang DS, et al. CDC20 overexpression predicts a poor prognosis for patients with colorectal cancer. *J Transl Med* 2013;11:142.
159. Ding ZY, Wu HR, Zhang JM, et al. Expression characteristics of CDC20 in gastric cancer and its correlation with poor prognosis. *Int J Clin Exp Pathol* 2014;7:722–7.
160. Marucci G, Morandi L, Magrini E, et al. Gene expression profiling in glioblastoma and immunohistochemical evaluation of IGFBP-2 and CDC20. *Virchows Archiv* 2008;453:599–609.
161. Li L, Lei Q, Zhang S, et al. Screening and identification of key biomarkers in hepatocellular carcinoma: evidence from bioinformatic analysis. *Oncol Rep* 2017;38:2607–18.
162. Li H, Jiang X, Zhu S, et al. Identification of personalized dysregulated pathways in hepatocellular carcinoma. *Pathol Res Pract* 2017;213: 327–NaN.
163. Li J, Gao JZ, Du JL, et al. Increased CDC20 expression is associated with development and progression of hepatocellular carcinoma. *Int J Oncol* 2014;45:1547–55.
164. Gu Y, Lu L, Wu L, et al. Identification of prognostic genes in kidney renal clear cell carcinoma by RNAseq data analysis. *Mol Med Rep* 2017;15: 1661–7.
165. Kato T, Daigo Y, Aragaki M, et al. Overexpression of CDC20 predicts poor prognosis in primary non-small cell lung cancer patients. *J Surg Oncol* 2012;106:423–30.
166. Yi J, Wei X, Li X, et al. A genome-wide comprehensive analysis of alterations in driver genes in non-small-cell lung cancer. *Anticancer Drugs* 2018;29:10–8.
167. Shi R, Sun Q, Sun J, Wang X, Xia W, Dong G, Wang A, Jiang F, Xu L. Cell division cycle 20 overexpression predicts poor prognosis for patients with lung adenocarcinoma. *Tumour Biol* 2017; 39: 1010428317692233, 101042831769223.
168. Lub S, Maes A, Maes K, et al. Inhibiting the anaphase promoting complex/cyclosome induces a metaphase arrest and cell death in multiple myeloma cells. *Oncotarget* 2016;7:4062–76.
169. Borges DP, Dos Santos AWA, Paier CRK, et al. Prognostic importance of aurora kinases and mitotic spindle genes transcript levels in Myelodysplastic syndrome. *Leuk Res* 2018;64:61–70.
170. Genga KR, Filho FD, Ferreira FV, et al. Proteins of the mitotic checkpoint and spindle are related to chromosomal instability and unfavourable prognosis in patients with myelodysplastic syndrome. *J Clin Pathol* 2015;68:381–7.
171. Heredia FF, de Sousa JC, Ribeiro Junior HL, et al. Proteins related to the spindle and checkpoint mitotic emphasize the different pathogenesis of hypoplastic MDS. *Leuk Res* 2014;38:218–4.
172. Moura IM, Delgado ML, Silva PM, et al. High CDC20 expression is associated with poor prognosis in oral squamous cell carcinoma. *J Oral Pathol Med* 2014;43:225–31.
173. Li D, Zhu J, Firozi PF, et al. Overexpression of oncogenic STK15/BTAK/aurora a kinase in human pancreatic cancer. *Clin Cancer Res* 2003; 9:991–7.
174. Chang DZ, Ma Y, Ji B, et al. Increased CDC20 expression is associated with pancreatic ductal adenocarcinoma differentiation and progression. *J Hematol Oncol* 2012;5:15.
175. Mao Y, Li K, Lu L, et al. Overexpression of Cdc20 in clinically localized prostate cancer: relation to high Gleason score and biochemical recurrence after laparoscopic radical prostatectomy. *Cancer Biomarkers* 2016;16:351–8.
176. Ouellet V, Guyot MC, Le Page C, et al. Tissue array analysis of expression microarray candidates identifies markers associated with tumor grade and outcome in serous epithelial ovarian cancer. *Int J Cancer* 2006;119:599–607.
177. Choi JW, Kim Y, Lee JH, et al. High expression of spindle assembly checkpoint proteins CDC20 and MAD2 is associated with poor prognosis in urothelial bladder cancer. *Virchows Archiv* 2013; 463:681–7.
178. Barlin JN, Zhou QC, Leitao MM, et al. Molecular subtypes of uterine leiomyosarcoma and correlation with clinical outcome. *Neoplasia* 2015;17: 183–9.
179. Ryan SD, Britigan EM, Zasadil LM, et al. Up-regulation of the mitotic checkpoint component Mad1 causes chromosomal instability and resistance to microtubule poisons. *Proc Natl Acad Sci USA* 2012;109:E2205–14.
180. Pinto M, Soares MJ, Cerveira N, et al. Expression changes of the MAD mitotic checkpoint gene family in renal cell carcinomas characterized by numerical chromosome changes. *Virchows Archiv* 2007;450:379–85.
181. Nishigaki R, Osaki M, Hiratsuka M, et al. Proteomic identification of differentially-expressed genes in human gastric carcinomas. *Proteomics* 2005;5:3205–13.

182. Osaki M, Inoue T, Yamaguchi S, et al. MAD1 (mitotic arrest deficiency 1) is a candidate for a tumor suppressor gene in human stomach. *Virchows Archiv* 2007;451:771–9.
183. Nam CW, Park NH, Park BR, et al. Mitotic checkpoint gene MAD1 in hepatocellular carcinoma is associated with tumor recurrence after surgical resection. *J Surg Oncol* 2008;97: 567–71.
184. Li D, Meng Q, Zhang H, et al. Mitotic arrest deficient-like 1 is correlated with poor prognosis in small-cell lung cancer after surgical resection. *Tumour Biol* 2016;37:4393–8.
185. Sun Q, Zhang X, Liu T, et al. Increased expression of mitotic arrest deficient-like 1 (MAD1L1) is associated with poor prognosis and insensitive to Taxol treatment in breast cancer. *Breast Cancer Res Treat* 2013;140:323–0.
186. Li GQ, Zhang HF. Mad2 and p27 expression profiles in colorectal cancer and its clinical significance. *World J Gastroenterol* 2004;10: 3218–20.
187. Wang L, Yin F, Du Y, et al. MAD2 as a key component of mitotic checkpoint: a probable prognostic factor for gastric cancer. *Am J Clin Pathol* 2009;131:793–801.
188. Zhang SH, Xu AM, Chen XF, et al. Clinicopathologic significance of mitotic arrest defective protein 2 overexpression in hepatocellular carcinoma. *Hum Pathol* 2008;39:1827–34.
189. Furlong F, Fitzpatrick P, O'Toole S, et al. Low MAD2 expression levels associate with reduced progression-free survival in patients with high-grade serous epithelial ovarian cancer. *J Pathol* 2012;226:746–55.
190. Kato T, Daigo Y, Aragaki M, et al. Overexpression of MAD2 predicts clinical outcome in primary lung cancer patients. *Lung Cancer* 2011;74: 124–31.
191. Shi YX, Zhu T, Zou T, et al. Prognostic and predictive values of CDK1 and MAD2L1 in lung adenocarcinoma. *Oncotarget* 2016;7: 85235–43.
192. Suraokar MB, Nunez MI, Diao L, et al. Expression profiling stratifies mesothelioma tumors and signifies deregulation of spindle checkpoint pathway and microtubule network with therapeutic implications. *Ann Oncology* 2014;25:1184–92.
193. Wang XG, Peng Y, Song XL, et al. Identification potential biomarkers and therapeutic agents in multiple myeloma based on bioinformatics analysis. *Eur Rev Med Pharmacol Sci* 2016;20:810–7.
194. Teixeira JH, Silva P, Faria J, et al. Clinicopathologic significance of BubR1 and Mad2 overexpression in oral cancer. *Oral Dis* 2015;21: 713–20.
195. Yu L, Guo WC, Zhao SH, et al. Mitotic arrest defective protein 2 expression abnormality and its clinicopathologic significance in human osteosarcoma. *APMIS* 2010;118:222–9.
196. Nakano Y, Sumi T, Teramae M, et al. Expression of the mitotic-arrest deficiency 2 is associated with chemotherapy resistance in ovarian serous adenocarcinoma. *Oncol Rep* 2012;28:1200–4.
197. Park PE, Jeong JY, Kim SZ, et al. MAD2 expression in ovarian carcinoma: different expression patterns and levels among various types of ovarian carcinoma and its prognostic significance in high-grade serous carcinoma. *Korean J Pathol* 2013;47:418–25.
198. Nakano Y, Sumi T, Morishita M, et al. Mitotic arrest deficiency 2 induces carcinogenesis in mucinous ovarian tumors. *Oncol Lett* 2012;3:281–6.
199. Chen F, Liu S, Zhou Y, et al. Mad2 overexpression is associated with high cell proliferation and reduced disease-free survival in primary gastrointestinal diffuse large B-cell lymphoma. *Hematology* 2016;21:399–403.
200. Hisaoka M, Matsuyama A, Hashimoto H. Aberrant MAD2 expression in soft-tissue sarcoma. *Pathol Int* 2008;58:329–3.
201. Fung MK, Cheung HW, Wong HL, et al. MAD2 expression and its significance in mitotic checkpoint control in testicular germ cell tumour. *Biochim Biophys Acta* 2007;1773:821–32.
202. Turner N, Lambros MB, Horlings HM, et al. Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. *Oncogene* 2010;29:2013–3.
203. Iwanaga Y, Chi YH, Miyazato A, et al. Heterozygous deletion of mitotic arrest-deficient protein 1 (MAD1) increases the incidence of tumors in mice. *Cancer Res* 2007;67:160–6.
204. Dai W, Wang Q, Liu T, et al. Slippage of mitotic arrest and enhanced tumor development in mice with BubR1 haploinsufficiency. *Cancer Res* 2004; 64:440–5.
205. Park I, Lee HO, Choi E, et al. Loss of BubR1 acetylation causes defects in spindle assembly checkpoint signaling and promotes tumor formation. *J Cell Biol* 2013;202:295–309.
206. Kalitsis P, Fowler KJ, Griffiths B, et al. Increased chromosome instability but not cancer predisposition in haploinsufficient Bub3 mice. *Genes Chromosomes Cancer* 2005;44:29–36.
207. Babu JR, Jeganathan KB, Baker DJ, et al. Rae1 is an essential mitotic checkpoint regulator that cooperates with Bub3 to prevent chromosome missegregation. *J Cell Biol* 2003;160:341–53.
208. Li M, Fang X, Wei Z, et al. Loss of spindle assembly checkpoint-mediated inhibition of Cdc20 promotes tumorigenesis in mice. *J Cell Biol* 2009;185:983–4.
209. Malureanu L, Jeganathan KB, Jin F, et al. Cdc20 hypomorphic mice fail to counteract de novo synthesis of cyclin B1 in mitosis. *J Cell Biol* 2010; 191:313–29.