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Tau mutations serve as a novel risk factor for cancer

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Abstract

In addition to its well-recognized role in neurodegeneration, tau participates in maintenance of genome stability and chromosome integrity. In particular, peripheral cells from patients affected by frontotemporal lobar degeneration carrying a mutation in tau gene (genetic tauopathies), as well as cells from animal models, show chromosome numerical and structural aberrations, chromatin anomalies, and a propensity toward abnormal recombination. As genome instability is tightly linked to cancer development, we hypothesized that mutated tau may be a susceptibility factor for cancer. Here we conducted a retrospective cohort study comparing cancer incidence in families affected by genetic tauopathies to control families. Additionally, we carried out a bioinformatics analysis to highlight pathways associated with the tau protein interactome. We report that the risk of developing cancer is significantly higher in families affected by genetic tauopathies, and a high proportion of tau protein interactors are involved in cellular processes particularly relevant to cancer. These findings disclose a novel role of tau as a risk factor for cancer, providing new insights in the various pathological roles of mutated tau.

Significance. This study reveals a novel role for tau as a risk factor for cancer providing new insights beyond its role in neurodegeneration

Introduction

Cancer arises from sequential accumulation of genomic alterations, including mutations in oncogenes and tumour suppressor genes (1). Over the years different models of oncogenesis have been proposed (2), in which an initiating event often caused by a mutation leads to genomic instability, comprising: (i) subtle sequence instabilities (base pair substitutions, deletions or insertions of few nucleotides), favoured by mutations in genes involved in the DNA nucleotide-excision and mismatch repair systems; (ii) chromosome instability (CIN), in particular aneuploidy, defined as loss or gain of whole chromosomes or large fragments thereof, that occurs following mutations in genes involved in cellular processes affecting correct chromosome segregation, such as chromosome condensation, chromatid cohesion, kinetocore assembly, centrosome replication/microtubule dynamics, DNA repair and cell cycle checkpoints (3-5). Overall, these types of genomic instability can cause changes in sequence, structure or allelic number of tumour genes, leading cancer cells to acquire functional capabilities that allow them to survive, proliferate and metastasize.

Microtubule-associated proteins (MAPs) are defined as proteins promoting *in vivo* tubulin self-association into microtubules (MT). Other proteins interacting with MT have different functions such as MT destabilization, linking of various structures and motor properties. Most of these MT-related proteins play a role in mitotic spindle formation, ensuring correct chromosome segregation (6,7). Among MAPs, tau is the most relevant to the nervous system, being abundantly expressed in neurons. Deposition of insoluble filamentous forms of tau gives rise to tauopathy, a neuronal pathology that leads to dementia and atypical parkinsonian syndromes (8). Tau binds to interphasic cytoskeleton MT as well as to mitotic spindle MT (9-11). A mutated tau usually exhibits a reduced ability to bind to MT and to promote their assembly (12), altering MT dynamics (13,14). This can lead to an unstable mitotic spindle, from which chromosome mis-segregation can arise, causing aneuploidy.

In this regard, we demonstrated the consistent presence of aneuploidy in peripheral cells of patients affected by frontotemporal lobar degeneration (FTLD) due to autosomal dominant mutations in microtubule-associated protein tau (*MAPT*) gene, suggesting a role of tau in chromosome and genome stability (11,15). In addition, it has been reported that tau knock-out mice show chromosome mis-segregation and aneuploidy (16). Based on these studies and as previously suggested (3), we hypothesized that mutated tau can cause CIN resulting in aneuploidy. Aneuploidy is frequently observed in cancer cells, where it can lead to loss of heterozygosity of tumour suppressor genes or amplification of oncogenes, thereby contributing to tumorigenesis.

Moreover, a different type of CIN was observed in cells of our patients carrying mutated tau, i.e. structural chromosome aberrations (11,15), that may be ascribed to defects in DNA single- or double-strand break repair or in DNA-damage checkpoint (3), to telomeric DNA loss or telomerase defects (17), or to alterations in proteins contributing to chromatin stability, predisposing to a higher rate of DNA damage. Whereas there is no evidence of the involvement of tau in DNA repair systems (15) or in telomere preservation, a chaperone role of tau in protecting DNA from free radicals and heat stress damage has recently emerged (18,19), as well as a structural role of tau in chromatin stabilization (15).

Based on these findings, we hypothesized that CIN associated with tau mutations can lead to cancer. Thus, we propose a dual pathogenic role of tau mutations, in cancer and neurodegeneration.

Cancer and neurodegeneration have been proven to share alteration of some biological pathways such as cell cycle, apoptosis, ubiquitin-proteasome system (20,21). These pathways are usually differentially regulated in cancer and neurodegeneration due to the different nature of the impacted cells, that is, proliferating or post-mitotic, respectively. Cell cycle is dysregulated in cancer, where control over cell proliferation is inhibited, and in some forms of neurodegeneration, where abnormal re-entry of post-mitotic cells into the proliferating phase eventually leads to neuronal death via apoptosis (21,22). The tumor suppressor protein p53 plays a pro-apoptotic role and, while protecting the body from cancer, it promotes the aging phenotype through cellular loss. Its

deficiency by mutation can lead to higher cancer risk on one hand, and lower degree of neurodegeneration on the other (21). The ubiquitin-proteasome system, while impaired in neurodegenerative diseases and leading to misfolded protein accumulation, is upregulated in several types of cancer (21). A recent study showed that transcripts up-regulated in cancer are down-regulated in central nervous system diseases and *vice versa*. In line with this finding, a reduced risk for developing some types of cancer has been observed in patients affected by Parkinson's disease (PD) and Alzheimer's disease (AD) (23).

Metabolic dysregulation, oxidative stress, DNA damage and inflammation have been shown to be initiating events for both cancer and neurodegeneration (21). In addition, a number of genes (i.e. *ATM*, *PARK2* and *LRRK2*) are known to confer risk for both cancer and neurodegeneration. *ATM* plays a central role in cell division and DNA repair: homozygous mutations cause ataxia-teleangectasia, with degeneration of some cerebellar neurons, and predispose to high frequency to cancer, especially to lymphomas (24). *PARK2*, the most commonly mutated gene in autosomal recessive PD (25), is a well-known tumor-suppressor gene, whose loss of function mutations are associated with cancer (26). Similarly *LRRK2*, the most frequently mutated gene in late-onset PD has been linked to increased risk of some types of cancers (27).

Similarly to what happens for *ATM*, *PARK2* and *LRRK2* mutations, we here suggest that tau mutations may lead to both neurodegeneration and cancer. In fact, if tau is altered by a mutation, its role as MT-binding protein can lead to cytoskeleton disruption and tau deposits in neurodegeneration, and chromosome missegregation and aneuploidy in cancer; furthermore, its role as DNA-chaperone can lead to DNA damage and apoptosis in neurodegeneration and structural chromosome aberrations in cancer.

To verify the hypothesis that CIN associated with tau mutations can lead to cancer, we conducted a retrospective cohort study comparing cancer incidence in families affected by FTLD carrying tau mutations and reference families with superimposable pedigrees, and carried out a bioinformatics analysis of pathways associated with the tau protein interactome. We found that (i) members of

FTLD families have a significantly higher risk of developing cancer than members of control families, and (ii) 1/3rd of the tau interactors are involved in DNA damage recognition and repair, cell cycle checkpoints and phase transition, chromatin and telomere maintenance processes, and response to radiation stressors, supporting a role for tau in DNA protection/repair systems.

Materials and Methods

All the subjects participating in the study gave their written informed consent for using their clinical and genetic data for research purposes. All the procedures involving human subjects were done in accordance with the Helsinki Declaration of 1975. As this is a retrospective study, approval by an Ethics Committee was not required.

Pedigrees

We considered FTLD kindreds with a family history of disease and a mutation in the *MAPT* gene (detected by sequencing of exons 1, 9-13). We analysed 15 families bearing 7 different tau mutations. We designed this study as a retrospective cohort study, where tau-mutated families represented the "exposed cohort". For each tau-mutated family we collected data from 3 reference families with superimposable pedigrees, with a member born in the same year of the proband, of the same gender and native Italian region. To retrieve comparable informative data for all the tau-mutated families, the pedigrees were accurately investigated for the presence of cancer in: 1) the proband, 2) his/her siblings, 3) the parent whose family is affected by FTLD, 4) his/her siblings, 5) the grandparent affected by FTLD.

Clinical data were obtained from interviews with relatives and family doctors or directly from available clinical charts. For the reference families, the same methods were applied, except both paternal and maternal lines were investigated. Follow up was assessed by interviews for both tau-

mutated families and reference families. Where it was not possible to obtain an answer we used social security list to update vital status (2%).

Statistical methods

We compared cancer incidence for the tau-mutated families *versus* the reference families. In the analysis of tau-mutated families, we did not include the spouses and all the grandparents for which the information about presence of FTLD was not available (subjects in gray in Figure 1). We did not include 2 subjects affected by cancer whose genotype was wild-type (see legend of Figure 1).

The statistical analyses are based on the Cox proportional hazard model which specifies the hazard as $\lambda(t)=\lambda 0(t)\exp(\beta X)$, where $\lambda(t)$ is the hazard function for the event in question (cancer incidence). X is a vector of covariates, and β is a vector of coefficients to be estimated. The hazards for two participants with fixed covariate vectors Xi and Xj are $\lambda i(t)=\lambda 0(t)\exp(\beta Xi)$ and $\lambda j(t)=\lambda 0(t)\exp(\beta Xj)$, respectively. The hazard ratio (HR) is $\lambda i(t)/\lambda j(t)=\exp(\beta(Xi-Xj))$. To test the *null* hypothesis H0 that $\beta=0$, we used the likelihood ratio test. Since the Cox model assumes proportional hazards, this was tested by analysis of scaled Schoenfeld residuals, with associated *p*-values. When the hazard was suspected to be non-proportional over time, we performed additional analyses, substituting the conventional Cox β coefficient (for a given variable) with a time-dependent function $\beta(t)$ obtained by adding the smoothed scaled Schoenfeld residuals to the conventional β coefficient.

Factors known or thought to influence cancer incidence in the tau-mutated families were initially analysed by univariate Cox proportional hazard modelling to verify the effect on incidence in our retrospective cohort. Factors analysed were gender, year of birth and region of origin. We next ran multivariate Cox proportional hazard models to estimate HRs with 95% CIs of cancer events. The multivariate model was stratified (separate baseline hazard functions for each variable category within the model) by the same factors above to control for the possible confounding effects of these

variables on incidence. Time to event or end of follow-up was calculated from date of birth to date of cancer diagnosis or death or end of follow-up.

Data analysis was carried out using R-language.

Bioinformatics analysis of tau protein interactome

We built the tau weighted protein-protein interactome by extracting all the currently known tau's protein-protein interactors (PPIs), obtained from peer reviewed literature filtered and scored by our *in house* pipeline in a supervised manner (28). Briefly, we downloaded PPIs (in June-2017) from the following databases within the IMEX consortium: APID Interactomes, BioGrid, bhf-ucl, InnateDB, InnateDB-All, IntAct, mentha, MINT, InnateDB-IMEx, UniProt, and MBInfo by means of the "PSICQUIC" R package (version 1.15.0 by Paul Shannon, http://code.google.com/p/psicquic/). We converted Protein IDs to Swiss-Prot and Entrez gene ID; we removed TrEMBL, non-protein interactors (e.g. chemicals), obsolete Entrez and Entrez matching to multiple Swiss-Prot identifiers. All PPIs underwent quality control (QC) and filtering leading to the removal of: i) all the non-human taxid annotations; ii) all the annotations with multiple or none PubMed identifiers or no description of Interaction Detection Method. We then scored the interactions taking into consideration the following parameters: i) the number of different publications reporting the interaction; ii) the number of different methods reporting the interaction. We discarded all the interactors with a final score ≤ 2 because (still) not replicated. We performed Gene Ontology (GO) biological processes (BPs) enrichment analyses in g:Profiler (g:GOSt, http://biit.cs.ut.ee/gprofiler/; (29)) for the complete tau's interactome. Fisher's one-tailed test was used as statistical method for enrichment; SCS-threshold was applied as multiple testing correction; statistical domain size was only annotated genes; no hierarchical filtering was included. We grouped enriched GO-BP terms into custom-made "semantic classes". Data was handled, filtered and scored through *in-house* R scripts (https://www.r-project.org/) as described before (28). The

final network was visualized through the freely available Cytoscape 2.8.2 (30) software and analyzed through the network analysis plug-in.

Results

Epidemiology

The study population comprised 15 families bearing 7 different tau mutations (Fig. 1), whose FTLD-related pathogenic mechanisms are reported in Table 1. For four of these mutations, we demonstrated chromosome or genomic instability (11;15; Supplementary Table S1). As controls, for each tau-mutated family we selected three reference families with a member born in the same year of the proband, being of the same gender and native of the same Italian region. Demographics of both tau-mutated and reference families is shown in Table 2, while further details for reference families and regions of origin are reported in Supplementary Table S2.

All families were accurately investigated for the presence of cancer. Within the tau-mutated families, 24 subjects (15%) had cancer, while within the reference families 68 subjects (9%) had cancer. The mean age at diagnosis of subjects with cancer was 58 years, while the average age of dementia onset was 50 years. The types of cancer detected in tau-mutated and reference families are reported in Supplementary Table S3. A great variability was observed in both cohorts, showing no recurrence of a particular type of cancer even in tau-mutated families.

Factors known or thought to influence cancer incidence such as gender, year of birth and region of origin were initially analysed by univariate Cox proportional hazard model (Table 3A). The study showed that the tau-mutated families had significantly greater risk of cancer than the reference families (HR = 3.11), and, when the gender variable was assessed, the risk in females appeared to be greater than in males, though not significant (HR = 1.16). We then performed multivariate Cox proportional hazard model to estimate HRs with 95% CIs of cancer events. The model was stratified

by gender, year of birth and region of origin to control for potential confounding effects on incidence of cancer. The likelihood ratio test resulted in p = 0.0005, supporting robust association between the presence of a tau mutation and the development of cancer with HR = 3.72 (CI 95% 2.07 – 6.67), thus indicating a nearly 4-fold risk of developing cancer in tau-mutated families (Table 3B).

Bioinformatics analysis of tau protein interactome

We generated a two layers interactome for tau (Fig. 2). The first layer interactors (65 nodes) are directly connected to tau, whilst the second layer interactors (3132 nodes) represent the interactors of each first layer node thus diluting the seed centrality bias as previously reported (28). The global tau's interactome comprised a total of 3197 nodes and 5711 edges, with characteristic path length of 3.407 and average number of neighbors of 3.3 (Fig. 2A). To gather insight into the biological functions associated with tau's entire interactome we performed functional annotation analysis evaluating Gene Ontology-Biological Processes (GO-BPs) enrichment (Supplementary Table S4). Some of the biological functions enriched within tau's interactome were expected (e.g. cytoskeleton dynamics and transport). Nevertheless, nearly 1/3rd of the proteins contributing to the entire tau's interactome (989/3197) was directly involved in the enrichment of GO-BPs terms globally pointing to DNA METABOLISM and, particularly, to DNA damage, stress response to radiation, DNA damage checkpoint and repair, and cell death after DNA damage; additionally, we found terms related to CELL CYCLE, particularly, indicating cell cycle checkpoints and chromosome segregation, and CHROMATIN, the latter pointing to processes such as histone and telomere maintenance (Table 4; Fig. 2B). Importantly, to assess the specificity of the enrichment reported above, we generated 25 random protein sets (by extracting a series of numbers using random permutation without replacement in R) with similar size to the tau's interactome and processed them through functional enrichment. Out of the 25 protein sets, only 8 (32%) led to a significant functional enrichment. Only 1/8 random protein set revealed 0.8% enriched terms that were similar

to those reported for tau. Considering the former (0.8%) against the latter (30.9%) (Supplementary Table S5), it follows that the specificity of tau's interactome is strong and unbiased.

Discussion

It is well established that tau, as a MT-binding protein, is a major player in neurodegenerative diseases also known as tauopathies, such as FTLD and AD. Cytoplasmic abnormal tau deposits represent a burden to neurons and glial cells, whilst toxic soluble tau oligomers are now being envisaged as responsible for the neuronal dysfunction and death (8,31).

However, other lines of evidence suggest that tau may be also involved in other functions. Nuclear and nucleolar localizations of tau were first described several years ago (32-34) and, more recently, a role of tau in ribosome biogenesis was suggested (35). We confirmed the nuclear and peri-chromosomal localization of tau and, in addition, discovered that FTLD patients bearing the P301L tau mutation had several numerical and structural chromosome aberrations and chromatin defects in their peripheral blood lymphocytes and fibroblasts (11).

A number of observations argued for a link between mutated tau and chromosome aberrations: 1) tau's physical association with mitotic spindle, thus possibly regulating correct chromosome segregation; 2) tau's nuclear localization and its physical interaction with the chromatin (36); 3) tau's ability to protect DNA *in vitro* (37). Additionally, a link between tau and genome integrity was evidenced when tau was shown to translocate from the cytoplasm to the nucleus during cellular stress and to protect DNA from stress-induced DNA breaks (19). Furthermore, in an *in vivo* mouse model of heat stress, tau was shown to protect genomic DNA and also RNA from oxidative damage (38), whilst in a tau knock-out mouse model, the absence of tau caused disruption of the neuronal peri-centromeric heterochromatin, which showed an abnormal accumulation of DNA breaks (39).

Our group examined peripheral cells of FTLD patients carrying different tau mutations and demonstrated chromosome aberrations, as well as a tendency to abnormal recombination events, indicating genome instability (15). All the more, in two mouse models of genetic tauopathy, we detected a higher level of aneuploidy than in control mice (40). More recently, in a *Drosophila melanogaster* model of tauopathy, mitotic spindle anomalies and aneuploidy were observed after overexpressing wt tau (41).

Aneuploidy is a condition often associated with cancer. An aneuploid karyotype can or cannot promote cancer depending on its inherent imbalance of oncogenes or tumor suppressor genes, and other genes controlling cell viability and fitness. It has been proposed that in the harsh cell environment experimented by cancer cells, aneuploidy may conceivably confer an advantage and promote cancer cells' survival and proliferation (42). Similar considerations apply to structural chromosome aberrations.

We therefore hypothesized that tau mutations might predispose to cancer. We had access to a retrospective cohort of families affected by genetic tauopathies (tau-mutated families) and investigated the potential link between tau mutations and cancer. We surveyed the presence of any type of cancer in subjects within tau-mutated families. In parallel, we collected reference families to compare cancer incidence. For the period of time when most of the tau-mutated families' subjects had lived there was not any national or regional cancer registry available, therefore, in the setting of retrospective studies, the most appropriate analysis model we could choose was the cohort model. Multivariate analysis correcting for the possible confounding factors showed that the presence of tau mutation raises the risk of developing cancer by 3.72 times, assigning to tau mutation a prominent role as a risk factor for cancer. If we accept the definition of moderate risk (in terms of disease incidence) as a risk two to four times as high as in the general population, we can affirm that *MAPT* mutations, as in the case of other genes such as *ATM* or *CHEK2* (43), represent a moderate risk factor for cancer.

This finding was further supported by our computational analysis where we applied a systems biology approach focused on the tau protein interactome on the basis of the guilt by association principle (i.e. the unknown function of protein A can be inferred via the known function of protein B if A and B interact) (44). Functional annotation analysis of the *in silico* model of tau's interactome showed that over 1/3rd of tau interactors was directly involved in functions such as DNA damage, response to radiation stressors, DNA damage checkpoint, repair and cell death, cell cycle checkpoints and chromatin maintenance, processes that are arguably associated with cancer; this is cross-supportive with previous computational work when considering tau's co-expression or PPI-networks analyses in FTLD (28,45).

Bridging the *in silico* and functional data, and considering in particular the cell cycle checkpoints, it has in fact been shown, in a *Drosophila* model of tauopathy, that human mutations cause neurodegeneration by abnormally activating the cell cycle in post-mitotic neurons (46), or can induce heterochromatin relaxation, DNA damage and widely altered gene expression with cell cycle activation (47,48). While abnormal neuronal cell-cycle reentry is now accepted as a phenomenon associated with neurodegeneration (22,49), the ability of mutated tau to activate the cell cycle in different tissues should be taken into account as a possible risk factor for abnormal cell proliferation, linking cancer and neurodegeneration. On the other hand, the DNA damage checkpoint mediated by ATM and p53 appears to be protective in mouse and *Drosophila* models of tauopathy, again linking neurodegeneration and cancer (50).

Figure 3 synthetically illustrates the possible mechanisms through which mutated tau can increase the risk of developing cancer. In particular, chromosome missegregation, leading to aneuploidy (Fig. 3A), chromatin damage, causing structural chromosome aberrations (Fig. 3B), and abnormal cell cycle activation (Fig. 3C) are depicted.

The types of tumors that we detected in tau-mutated families were variable, ranging from haematological (e.g. leukemias) to solid, from common (lung, breast, colorectal cancer) to rare, from benign (e.g. leiomyoma) to malignant, from strictly epithelial to teratomas (e.g., ovarian

teratoma). This spectrum suggests that tau mutations may represent a risk factor predisposing to genomic instability with no tissue specificity, as previously suggested by the presence of different and not recurrent types of chromatin and chromosome aberrations in FTLD patients (15).

By interrogating publicly available RNA/protein expression databases, we verified that tau was detected in almost all evaluated tissues (in both normal as well as cancerous tissues/cell lines) (Supplementary Table S6). It may be also worth considering that, due to the metastatic nature of many cancers, the tissue where a cancer is detected – as indicated in Supplementary Table S2 –may not be the primary tumor site and may thus be independent of tau's site-specific expression.

As shown in Figure 1, as well as in Supplementary Table S2, there are some families without cancer-affected subjects. As tau mutations do not represent a causative but a risk factor for cancer, it may be that not every subject carrying the mutation will develop cancer, and by pure chance it is possible that in a tau-mutated family there should not be cancer-affected subjects. This is the case of Fam11, Fam15 and Fam8, whereas other families carrying the same mutation show cancer-affected subjects; this may also be the case of the V337M mutation. However we do not exclude that some mutations may be less cancer-predisposing than others, depending on their position in the protein or their amino-acid change, which may affect to a minor degree the microtubule-binding capacity or the DNA-chaperone ability, involved in the cancer development. This may for example be the case of the V337M mutation.

In summary, we here show that tau's functions go beyond assuring MT stability, as we demonstrate its nuclear involvement and association with genome stability and increased risk for cancer. This is a novel concept for tau's biology and, in line with other reports tying cancer and neurodegeneration (20,21), it might prove critical for a better understanding of both cancer and neurodegeneration (i.e. tauopathies) etiologies. As such it is warranted to further explore tau-associated molecular mechanisms as a mean for untangling manifold disorders.

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Table 1. Pathogenetic mechanisms of tau mutations

Number of families in this study	**Tau mutation**	**Reported pathogenetic mechanisms[a]**	**Chromosome aberrations**
1	N279K	tau isoform imbalance; increase of tau self-aggregation.	n.a.
1	delN296	reduction of MT polymerization; slower kinesin translocation along MT	yes[b]
8	P301L	reduction of MT polymerization; increase of tau self-aggregation; slower kinesin translocation along MT	yes[b,c]
2	IVS10+16C>T	tau isoform imbalance	yes[b]
1	V337M	reduction of MT polymerization; increase of tau self-aggregation	n.a.
1	V363A	reduction of MT polymerization; oligomer production	yes[d]
1	T427M	n.a.	n.a.

n.a. = not available. [a]Reference 12; [b]Reference 15; [c]Reference 11; [d]this study, Supplementary Table S1.

Table 2. Cohort demographic

	N° of subjects of the whole cohort	N° of subjects of tau-mutated families	N° of subjects of reference families
Total subjects	879	162	717
Subjects with dementia		68	
Subjects with cancer		16	68
Subjects with dementia and cancer		8	
Gender			
M	473	94	379
F	406	68	338
Period			
1877-1916	107	5	102
1917-1936	210	24	186
1937-1956	232	46	186
1957-1996	219	50	169
1997-2013	111	37	74

Table 3A. Univariate analysis HRs and 95% CI for cancer risk

	HR (95% CI)
	univariate
Tau exposure	3.11 (1.93 – 5.31)
Gender	1.16 (0.76 - 1.74)

Table 3B. Multivariate analysis HRs and 95% CI for cancer risk

HR (95% CI)
multivariate
3.72 (2.07 – 6.67)*

*Multivariate stratified by gender, year of birth and region of origin

Table 4. Summary of Gene Ontology - Biological Processes (GO-BPs) terms enriched within the tau interactome and generally relevant to cancer.

p-value	term ID	t name	t depth	Semantic class
5.88E-09	GO:0071156	regulation of cell cycle arrest	6	cell cycle
3.14E-08	GO:0071158	positive regulation of cell cycle arrest	6	cell cycle
1.49E-07	GO:0044819	mitotic G1/S transition checkpoint	9	cell cycle - checkpoint
5.53E-07	GO:0072413	signal transduction involved in mitotic cell cycle checkpoint	7	cell cycle - checkpoint
9.28E-08	GO:0072395	signal transduction involved in cell cycle checkpoint	6	cell cycle - checkpoint
7.13E-12	GO:0007093	mitotic cell cycle checkpoint	6	cell cycle - checkpoint
1.85E-15	GO:0000075	cell cycle checkpoint	5	cell cycle - checkpoint
5.58E-06	GO:0000079	regulation of cyclin-dependent protein serine/threonine kinase activity	6	cell cycle - enzyme
1.37E-13	GO:2000045	regulation of G1/S transition of mitotic cell cycle	8	cell cycle - phase transition
3.28E-08	GO:2000134	negative regulation of G1/S transition of mitotic cell cycle	8	cell cycle - phase transition
7.20E-06	GO:0010389	regulation of G2/M transition of mitotic cell cycle	8	cell cycle - phase transition
2.37E-03	GO:0010972	negative regulation of G2/M transition of mitotic cell cycle	8	cell cycle - phase transition
4.68E-02	GO:0010824	regulation of centrosome duplication	7	cell cycle - segregation/cytokinesis
6.12E-12	GO:0000280	nuclear division	6	cell cycle - segregation/cytokinesis
3.78E-10	GO:0007088	regulation of mitotic nuclear division	6	cell cycle - segregation/cytokinesis
2.11E-09	GO:0051783	regulation of nuclear division	6	cell cycle - segregation/cytokinesis
7.23E-03	GO:0033045	regulation of sister chromatid segregation	6	cell cycle - segregation/cytokinesis
3.37E-02	GO:0051988	regulation of attachment of spindle microtubules to kinetochore	6	cell cycle - segregation/cytokinesis
3.58E-02	GO:0032465	regulation of cytokinesis	6	cell cycle - segregation/cytokinesis
6.04E-04	GO:0046605	regulation of centrosome cycle	6	cell cycle-cytoskeleton
6.51E-03	GO:0090307	mitotic spindle assembly	6	cell cycle-cytoskeleton
3.93E-10	GO:0032206	positive regulation of telomere maintenance	7	chomatin - telomere
1.54E-08	GO:0032212	positive regulation of telomere maintenance via telomerase	7	chomatin - telomere
6.17E-03	GO:0032205	negative regulation of telomere maintenance	7	chomatin - telomere
8.52E-14	GO:0032200	telomere organization	6	chomatin - telomere
1.04E-03	GO:0090671	telomerase RNA localization to Cajal body	6	chomatin - telomere
7.16E-03	GO:1904814	regulation of protein localization to chromosome, telomeric region	6	chomatin - telomere
1.04E-03	GO:0090672	telomerase RNA localization	5	chomatin - telomere
3.02E-09	GO:1905269	positive regulation of chromatin organization	7	chromatin - organisation
1.99E-07	GO:0031056	regulation of histone modification	7	histone
2.79E-07	GO:0031058	positive regulation of histone modification	7	histone
4.40E-03	GO:0070932	histone H3 deacetylation	8	histone - acetylation
3.25E-02	GO:0035065	regulation of histone acetylation	8	histone - acetylation
1.12E-07	GO:0016572	histone phosphorylation	7	histone - phosphorylation
5.64E-16	GO:0042770	signal transduction in response to DNA damage	6	DNA metabolism - damage
1.50E-03	GO:0006975	DNA damage induced protein phosphorylation	6	DNA metabolism - damage
6.84E-06	GO:0042771	intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	8	DNA metabolism - damage - cell death
2.82E-04	GO:1902229	regulation of intrinsic apoptotic signaling pathway in response to DNA damage	8	DNA metabolism - damage - cell death
2.48E-14	GO:0008630	intrinsic apoptotic signaling pathway in response to DNA damage	7	DNA metabolism - damage - cell death
3.27E-03	GO:1902230	negative regulation of intrinsic apoptotic signaling pathway in response to DNA damage	7	DNA metabolism - damage - cell death
1.49E-07	GO:0031571	mitotic G1 DNA damage checkpoint	10	DNA metabolism - damage - checkpoint
5.48E-06	GO:0006977	DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest	10	DNA metabolism - damage - checkpoint
5.53E-07	GO:1902402	signal transduction involved in mitotic DNA damage checkpoint	9	DNA metabolism - damage - checkpoint
1.75E-06	GO:0072431	signal transduction involved in mitotic G1 DNA damage checkpoint	9	DNA metabolism - damage - checkpoint
4.56E-05	GO:0006289	nucleotide-excision repair	7	DNA metabolism - repair
2.38E-02	GO:0006302	double-strand break repair	7	DNA metabolism - repair
1.43E-06	GO:0034644	cellular response to UV	7	response to stimulus - stress - radiation
2.51E-03	GO:0071480	cellular response to gamma radiation	7	response to stimulus - stress - radiation

For each semantic class the top GO terms are reported (based on t-depth).

Figure Legends

Figure 1. **Pedigrees of FTLD families**. Black symbols indicate subjects affected by FTLD, red symbols subjects affected by cancer and blue symbols subjects affected by both diseases. Gray symbols represent the spouse parents or grandparents (who do not belong to the family carrying FTLD); note that both grandparents were considered spouses in families 3, 6, 11 and 14, because information about which grandparent carried FTLD was not available. Diagonal lines indicate the deceased, arrows the probands. Smaller symbols represent subjects deceased within few days from birth, stillborn or aborted. Asterisks indicate subjects whose *MAPT* genotype was determined as wild-type (not included in the statistical analysis).

Figure 2. Tau protein interactome. **a** Tau (*MAPT*), in red, is used as seed to download direct protein interactors (first layer tau's interactome); direct interactors of tau are used to download direct protein interactors of the first layer nodes generating the second layer tau's interactome. **b** Updated version of A where all the first and second layer interactors (989 nodes) of tau associated with GO-BPs related to DNA damage, cell cycle checkpoints and chromatin/telomere maintenance are highlighted in dark blue.

Figure 3. Tau mutations increase the risk of cancer. a Mutated tau alters microtubule dynamics producing an unstable mitotic spindle, which in turn leads to chromosome missegregation. **b** Mutated tau fails to protect chromatin structure and DNA integrity, producing chromosome structural aberrations. **c** Mutated tau induces an aberrant cell cycle activation. All these pathological events can increase the risk of developing cancer.

Figure 1

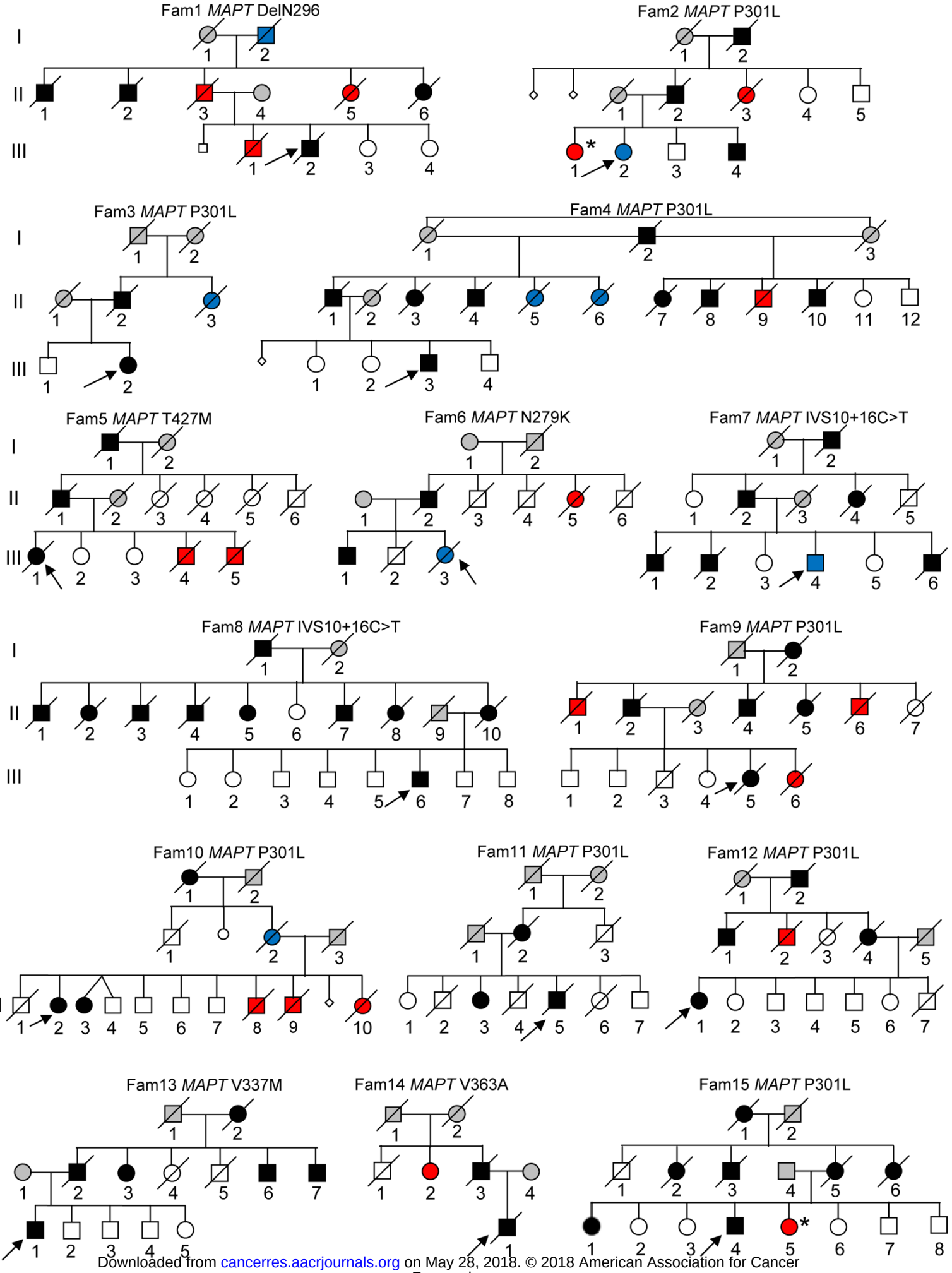


Figure 2

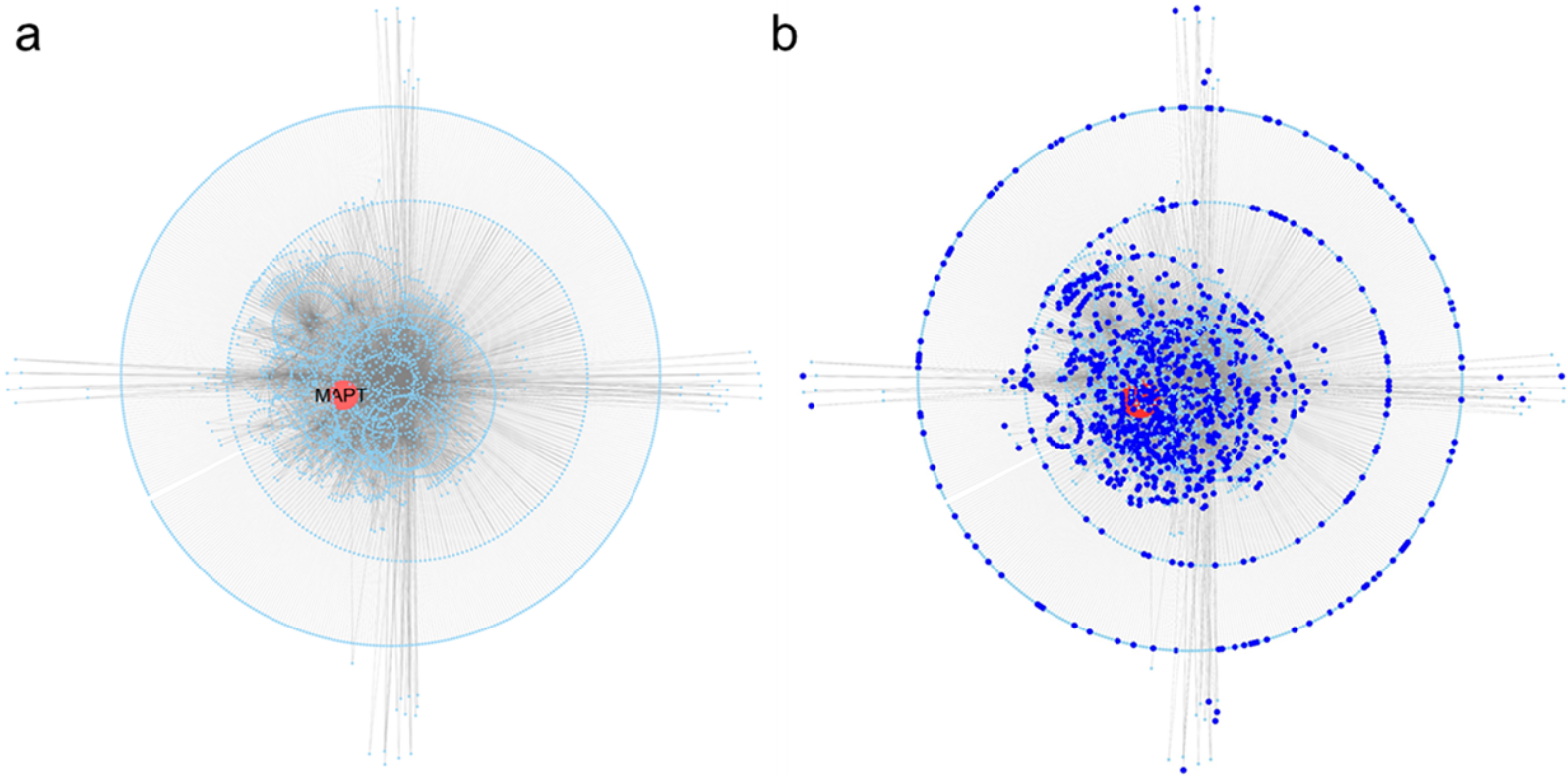
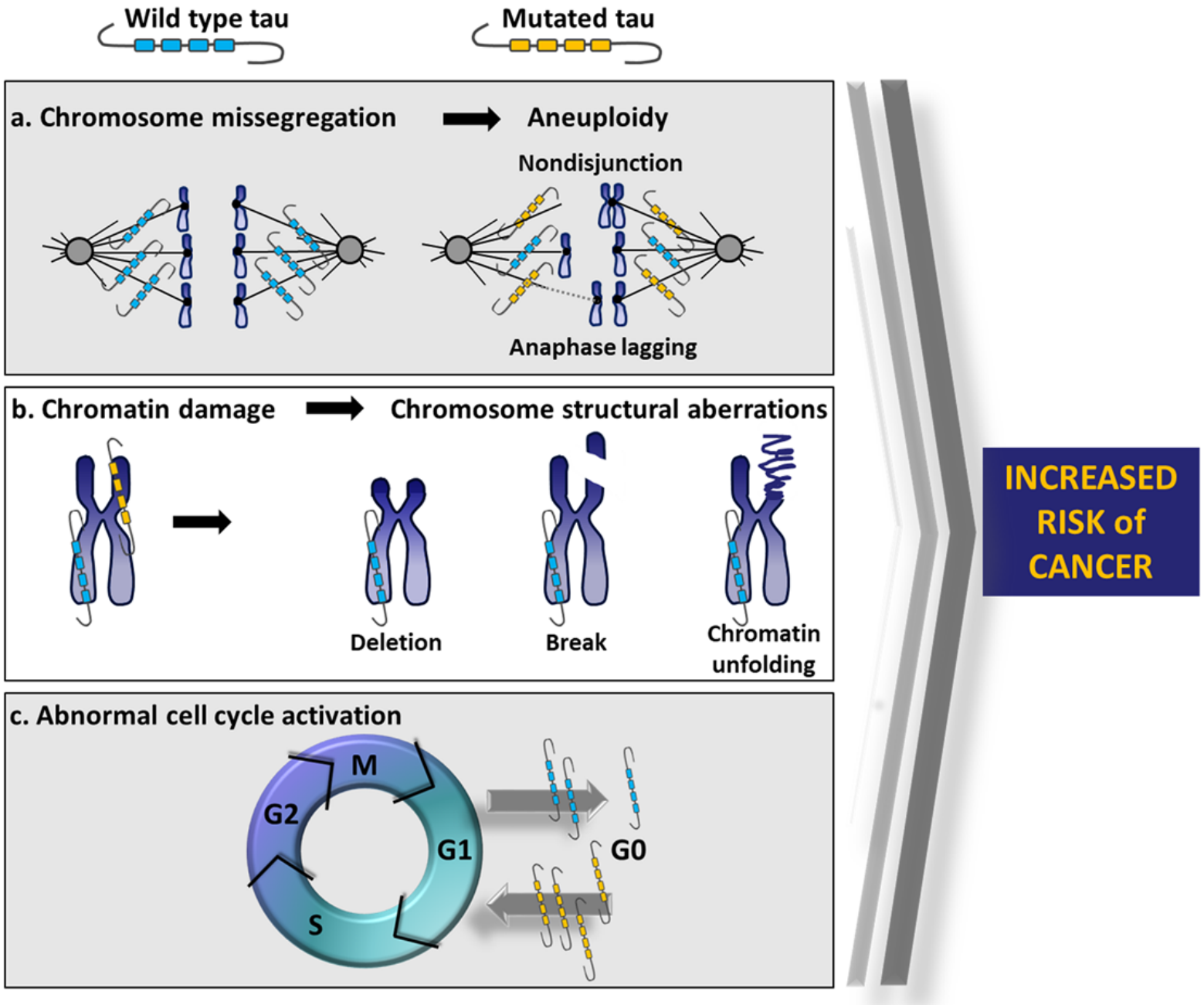


Figure 3



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