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Lack of Association Between Cancer History and *PARKIN* Genotype: a Family Based Study in *PARKIN*/Parkinson's Families

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Abstract

A number of publications have attributed a tumor suppressive (TS) function to *PARKIN*, a gene associated with recessive familial early-onset Parkinson's disease (EOPD). Discoveries of *PARKIN* deletions and point mutations in tumors, functional studies, and data from mouse models have been presented to support the hypothesis. We have asked whether *PARKIN* mutations are associated with history of cancer in humans. We interviewed 431 participants who were screened for *PARKIN* mutations, including 149 EOPD cases and their family members, who were unaware of mutation status. We found no significant difference in self-reported history of cancer among carriers of one or two *PARKIN* mutations and non-carriers, odds ratio 0.75 (95% confidence interval 0.27-1.83). In particular, no increase in cancer history was seen among homozygous and compound heterozygous mutation carriers compared to non-carriers. Therefore, we hypothesize that published studies attributing TS capability to *PARKIN* merit further exploration and we present a reevaluation of these data with respect to patterns of mutation frequencies in normal and cancer cells. We conclude that although *Parkin* may exert a suppressive effect in mice, further studies are required prior to assigning a TS function to *PARKIN* in humans.

Keywords

Tumor suppressor; PARKIN - Early-onset Parkinson's cohort; cancer risk

Several research publications (Cesari et al., 2003; Fujiwara et al., 2008; Poulogiannis et al., 2010; Veeriah et al., 2010), as well as some recent commentaries (Alderton, 2010; Garber, 2010) have attributed a tumor suppressive (TS) function to *PARKIN* (*PARK2*), a very large

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gene in chromosome band 6q26 responsible for a large proportion of recessive familial early-onset Parkinson's disease (EOPD) (reviewed in Marder et al., 2010). The studies in humans all present analyses of *PARKIN* deletions in cancers, and indeed it was the finding of unexpectedly high frequencies of loss of heterozygosity and/or deletions in the tumor samples which first prompted the hypothesis that *PARKIN* is a tumor suppressor gene (Cesari et al., 2003). A number of other findings have been interpreted as supporting the TS hypothesis, including increased growth rate of *PARKIN*-deficient cells in vivo and in culture (Poulogiannis et al., 2010; Veeriah et al., 2010) perhaps due to increased levels of cyclin E (Veeriah et al., 2010), a ubiquitination target of *PARKIN*; the presence of somatic point mutations in cancers (Poulogiannis et al., 2010; Veeriah et al., 2010); tumors in *Parkin* deficient mice (Fujiwara et al., 2008) and an increase in tumors in *Apc-Parkin* double mutant mice compared to *Apc* mutants alone (Poulogiannis et al., 2010). To determine whether a history of cancer is associated with the presence of mutations in *PARKIN* we interviewed probands with Parkinson disease and their family members participating in the CORE PD Study (NS036630; Marder et al., 2010). In light of our findings we discuss the literature on deletions, point mutations and functional studies of *PARKIN* in the human and mouse systems.

MATERIALS AND METHODS

Self-reported history of cancer was collected from 431 participants in a genetic study of EOPD (n=149) and their family members (n=282), the Consortium on Risk for Early-Onset PD study (CORE-PD; Marder et al., 2010). All participants were genotyped for *PARKIN* mutations, and included 30 carriers of two (homozygotes or compound heterozygotes), 114 *PARKIN* mutation heterozygotes and 287 non-carriers. Participants were asked about history of cancer (yes/no), from a pre-specified list. Since this was not a primary aim of the study, information on age at diagnosis of cancer was not obtained. None of the participants (or study researchers) was provided with results of the genetic analysis. Institutional review boards of all sites approved the protocol.

RESULTS

Results are summarized in Table 1. There was no significant difference in age and gender between mutation carriers and non-carriers, but as expected, carriers of *PARKIN* mutations were more likely to have PD than non-carriers. Six of 144 individuals who carried any *PARKIN* mutation (single or two) reported history of cancer compared with 17 of 287 non-carriers (see Table 1); odds ratio (OR) 0.75 (95% confidence interval 0.27-1.83). Of particular note, only one of the 30 individuals who carried two mutations, a 54-year-old carrier of an exon 4 deletion and Arg275Trp, reported history of cancer, similar to the frequency among the heterozygotes, and to the expected incidence of about 3% in the general population up to age 54 (Schouten et al., 1994). Since cancer incidence increases with age, we separately analyzed those over 60 at the time of assessment. Again we did not find a difference between carriers and non-carriers with 2/30 (6.7%) vs. 9/83 (11%) reporting a history of cancer, respectively ($p > 0.5$).

DISCUSSION

The CORE PD study is the single largest study of *PARKIN* in EOPD using a common assessment battery (Alcalay et al., 2010). Considering the size of our cohort, the frequency of self-reported cancer in the non-carrier group, and a Type I error of 0.05, our study had 80% power to detect a significantly different prevalence at the 0.05 level of self-reported cancer in the *PARKIN* mutation carrier group had the frequency in *PARKIN* mutation carriers been lower than 0.003 (<1/144 participants with *PARKIN* who had history of

cancer) or higher than 0.112 (>16/144 participants with *PARKIN* who had history of cancer). The fact that only 6 of the 144 *PARKIN* carriers reported history of cancer (less than 16) indicates either that cancer history frequency is not different between carriers and non-carriers, or that a Type-2 error has occurred because the association is weak.

It is acknowledged that there are a number of limitations in this dataset. Self-reporting of cancer history is not as reliable as the ‘gold standard’ of obtaining information from cancer registries, and we did not obtain confirmation of self-reporting through tissue banks; nevertheless, this form of data collection has been found to be a good substitute when the alternative is not feasible (Klein et al., 2010), especially when a specific list of cancers is given to the respondents as was the case here. Of note, melanomas were overrepresented amongst probands with PD (4/10 tumors vs. 2/13 among non-PD individuals), which is as expected if self-reporting was accurate. Within this subset of tumors there was again no evidence of association with *PARKIN* mutations (Table 1). Further limitations include the lack of cancer history in deceased family members (who were not genotyped) and the lack of age-of-onset of cancer, which could be a source of bias because the cohort was early-onset PD, and therefore relatively young. However, as noted in the Results section, there was no increase in cancer frequency among those over age 60. Overall, in spite of the caveats listed, we found no evidence that carrying *PARKIN* mutations confers a higher risk for cancer. How can we reconcile this conclusion with the mass of data in the literature implicating *PARKIN* as a TSG?

Deletions

Deletion frequencies of *PARKIN* sequences of up to 50%, clustering around exons 2- 4 have been described in many tumor types (Cesari et al., 2003; Fujiwara et al., 2008; Poulogiannis et al., 2010; Veeriah et al., 2010). This apparent association could be due to the postulated TS function of the gene, but an alternative possibility is the instability of this region of the human genome. In a recent genome-wide study of 440 trios and 1660 other individuals (Bradley et al., 2010a) a total of 13 deletion ‘hotspots’ were found in the genome, including one in the *PARKIN* gene. Independent germline deletions were found around exons 2-4 in about 0.75% of the population (half of which affected exons) representing a 100-fold increase over the average for the genome as a whole. In a separate study of only the coding sequences of *PARKIN* in healthy individuals almost exactly the same incidence was found, with 7 of 1686 (0.4%) being identified as carrying deletions in exons 1-4 and none in other exons (Kay et al., 2010). Although the deletion frequency in germ cells is much lower than the deletion frequency found in *PARKIN* in cancer it is probable that during cancer progression the inherent instability of the genomic region results in this high deletion frequency. We infer this because the other hotspots we discovered (Bradley et al., 2010a) are also subject to very high frequencies of deletions in cancer, independent of whether coding sequences are involved (Bradley et al., 2010b). For example, in a recent genome-wide study of clustering of deletions in several hundred cell lines (Bignell et al., 2010) clusters were found which coincide precisely with the most unstable hotspots we identified: on chromosomes 20 (centered at 15Mb), 9 (12Mb), 8 (5Mb) and 16 (6Mb), in addition to the region centered at exons 2-4 of *PARKIN*. The lack of involvement of coding sequences in the first four of these clusters led the authors to place these in an ‘unexplained’ category (Bignell et al., 2010) and they concluded that these deletions were ‘passengers’ rather than ‘drivers’ in cancer progression. We propose that the high deletion frequency in cancer lines in these four domains as well as in the *PARKIN* gene are a reflection of inherent genomic instability and may not be due to selective pressure against a tumor suppressor function of *PARKIN*.

Point mutations

A number of point mutations have been found in the *PARKIN* gene in cancers (see Table 2 for a summary), but to find these mutations more than 300 cancers have been screened. One study found no mutations in about 40 tumors (Cesari et al., 2003), another found one in 43 primary colorectal cancers (Poulogiannis et al., 2010; this was in addition to four mutations in five cell lines, two of which had two mutations each) and in yet another (Veeriah et al., 2010), 10 *PARKIN* point mutations were described in a collection of 242 cancers, together with four mutations in three cancer-derived cell lines including two in DLD1, which was also found to have two mutations in the study of Poulogiannis et al., 2010. To these can be added the results of the landmark paper in which most exons were sequenced in 22 cancers (Sjöblom et al., 2006) which reported 1307 mutations, none of which were in *PARKIN*. An estimate of the expected frequency of mutations in a given gene may be inferred from recent deep sequencing projects which show that cancer cells carry tens of thousands of mutations, including as many as 29 in *PARKIN* introns in one cancer (Pleasant et al., 2010a), and 11 in a cancer-derived cell line (Pleasant et al., 2010b). None of these mutations were in *PARKIN* exons. Since the coding exons represent 0.10% of the gene, this suggests an expectation of 6.9 coding mutations in the 340 (approximately) tumor samples just by chance, suggesting that the number of mutations discovered is not far out of the range expected in the absence of selective pressure. On the other hand, there is an enrichment of the order of 20-fold for mutations in cancer-derived cell lines: 11/340 vs. a total of 5 mutations in 8 cell lines, a difference which is extremely significant ($P = 7 \times 10^{-6}$ by Poisson analysis). The simplest explanation for this is that *PARKIN* mutations confer a phenotype strongly selected for in cell culture (see below), but not *in vivo*, so we conclude the evidence from point mutation frequencies does not support a TS function for *PARKIN* during tumorigenesis *per se*.

Functional studies

The functional studies cited in support of a tumor suppressor role for *PARKIN* comprised primarily the assessment of the effects on cyclin E levels through ubiquitination of cyclin E by the E3 ligase activity of *PARKIN* (Veeriah et al., 2010). Some of the consequences of knocking down *PARKIN* activity included enhanced cell growth rate and micronuclei formation, characteristics consistent with cyclin E upregulation in cell culture, but these observations in themselves do not constitute proof of *in vivo* tumor suppressor activity on the part of *PARKIN*. There is a plethora of E3 ubiquitin ligases each with a range of targets and conversely, it may be expected that each protein may be the ubiquitination target of several E3 ligases. This redundancy makes assignment of a crucial role to any particular ligase risky. For example, one ligase which is known to ubiquitinate cyclin E is FBXW7 which is indeed a potential tumor suppressor (Mao et al., 2004), but in addition to targeting cyclin E for degradation, it ubiquitinates a number of known proto-oncogenes (Welcker and Clurman, 2008) and it may be this multiplicity which provides sufficient growth control for a tumor suppressor effect.

Mouse model

In a study of cancer occurrence in *Parkin* homozygous knockout mice, one group (Fujiwara et al., 2008) reported hepatocellular carcinoma (HCC) in about 40% of 48 - 96 week- old mice, compared with none in the controls. In a second mouse model, *Apc-Parkin* double mutant mice were shown to have a dramatic four-fold increased frequency of intestinal tumors compared with mice carrying only the *Apc* mutation (Poulogiannis et al., 2010). While these results are convincing, their relevance for human cancer may be limited. HCC occurs spontaneously in some mouse strains, and its usefulness as a model for the human system has been questioned (Lee et al., 2005). In addition, the respective phenotypes in mice differ from those in human, as the *Apc* mutation targets intestine, not colon, and the *Parkin*

knockout does not predispose to any motor deficiency resembling PD in mice, and it is consequently not routinely used as a model for this disease (Perez and Palmiter, 2005). These difficulties may weaken the case the authors made for *PARKIN* involvement in human cancer, even though it remains clear that *PARKIN* mutations contribute to cancer in this model system.

In conclusion, we have directly tested the hypothesis that *PARKIN* is a TSG on the largest family-based PD study that genotyped all participants for *PARKIN* mutations. Then we critically reviewed the available literature that may support the TSG hypothesis; and raised alternative explanations that may explain these supporting data. Based on our findings we conclude that *PARKIN* is either not a TSG, or that the cells have a compensatory mechanism such that *PARKIN* mutations convey much lower risk for cancer than other genes whose TS function is widely accepted.

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Table 1Self-reported cancer history in the CORE-PD study, stratified by *PARKIN* mutation and PD status

	Wild type (n=287)	Heterozygotes (n=114)	Homozygotes/ compound HZ (n=30)	P- value
Age at evaluation, mean (SD)	50.6 (16.0)	47.5 (16.1)	50.4 (13.4)	0.195 ¹
Gender, number of women (%)	161 (56.1%)	60 (52.6%)	15 (50%)	0.708 ²
History of cancer, number (%)	17 (4.4%)	5 (5.9%)	1 (3.3%)	0.727 ²
Affected by PD				
Number	87 (30.3%)	37 (32.5%)	25 (83.3%)	
History of cancer, number (%)	7 (8%)	2 (3.7%)	1 (4%)	0.725 ²
Ages of affected	50, 51, 55, 57, 62, 62, 66	42, 48	54	
Types of cancer ³	Thyroid, lymphoma, uterine, prostate, melanoma (3)	Melanoma, cervical	Cervical	
Unaffected by PD				
Number	200 (69.7%)	77 (67.5%)	5 (16.7%)	
History of cancer, number (%)	10 (5%)	3 (3.9%)	0	0.819
Ages of affected	50, 51, 54, 64, 66, 70, 71, 75, 78, 82	60, 67, 70	N/A	
Types of cancer	Colon (2), Prostate (2), melanoma (2), breast (2), lung, cervical	Cervical, uterine, prostate	N/A	

¹: Analysis of variance (ANOVA)²Chi square

Table 2
Summary of searches in cancers and cell lines for point mutations affecting *PARKIN*

Study description	Number of Cancers	mutations in <i>PARKIN</i>	Number of cell lines	mutations in <i>PARKIN</i>	Reference
<i>PARKIN</i> only, exons	approx.				
	40	0	nd		Cesari et al., 2003
	242	10	4	3	Veeriah et al., 2010
Genome-wide, exons	43	1	5	4	Poulogiannis et al., 2010
	22	0	nd		Sjöblom et al., 2006
Genome-wide, genomic		29, only			
		1	in introns	nd	Pleasant et al., 2010a
				11, only	
	nd		1	in introns	Pleasant et al., 2010b

nd: not done